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EFFECT OF CHLORINATION ON HUMAN ENTERIC VIRUSES IN PARTIALLY TREATED WATER FROM THE POTOMAC RIVER ESTUARY

O. C. Liu, et al

Environmental Protection Agency
Narragansett, Rhode Island

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Study Report

Effect of Chlorination on Human Enteric Viruses in Partially Treated Water from the Potomac River Estuary

Prepared for the

United States Army Engineer Division, North Atlantic

as part of the

Northeastern United States Water Supply (NEWS) Study

by

O.C. Liu

Project Leader

F. McGowan

Project Officer

Environmental Protection Agency

Water Supply Programs Division

Northeastern Water Supply Laboratory

July 1971

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Participants in the project

Dr. B.H. Pringle, Director, Northeastern Water Supply Laboratory

Dr. N.A. Clarke, Chief, Analytical Methods Branch, Criteria & Standards

Dr. S.L. Chang, Chief, Etiology Section, Criteria & Standards

Dr. O.C. Liu, Principal Virologist, Northeastern Water Supply Laboratory

Miss H.R. Seraichekas, Virologist, Northeastern Water Supply Laboratory

Mr. E.W. Akin, Virologist, Gulf Coast Water Supply Laboratory

Mr. D.A. Brashear, Virologist, Northeastern Water Supply Laboratory

Mr. E.L. Katz, Chemist, Northeastern Water Supply Laboratory

Miss J.A. Barnick, Technician, Northeastern Water Supply Laboratory

Mr. R.J. Cipolla, Technician, Northeastern Water Supply Laboratory

Mr. P.F. Carey, Technician, Northeastern Water Supply Laboratory

Mr. D.L. Winslow, Media Room Supervisor, Northeastern Water Supply Laboratory

Mr. E.L. Strickland, Animal Room Technician, NE Water Supply Laboratory

Mrs. B.A. McFee, Laboratory Aid, Northeastern Water Supply Laboratory

Mrs. G.B. Calande, Secretary, Northeastern Water Supply Laboratory

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TABLE OF CONTENTS

Page

Summary and recommendations 1

Introduction 5

Background 9

(A) The potential health hazards from viruses in water . . . 9

(B) Epidemiology of waterborne virus diseases 14

(C) Efficiency of water treatments on elimination of virus . 18

(a) Storage 19

(b) Flocculation and sedimentation 21

(c) Filtration 22

(d) Disinfection 24

(i) Chlorination 25

(ii) Iodination 27

(iii) Ozonation 28

(iv) Radiation 28

Materials and methods 33

(A) Viruses 33

(B) Demand-free water 34

(C) Potomac estuarine water 34

(D) Glassware 35

(E) Chlorination experiment 35

(F) Viral assays 36

(a) Plaquing 36

(b) TCD_{50} 37
(c) LD_{50} 37
(G) Chlorination 38
(H) Chemical assays 38
(a) Sulfates 38
(b) Orthophosphate 39
(c) Nitrates 39
(d) Chlorine 39
(e) Chloride 40
(f) Sodium 40
(g) pH 40
(h) Alkalinity 40
(i) Turbidity 41
(j) Hardness 41
(k) Total dissolved solids 41
Experimental results 42
(A) Characterization of the Potomac water 42
(B) Determination of experimental parameters 42
(C) Determination of viral resistance to chlorine in Potomac water 47
(D) The relative resistance of 25 viruses to chlorine in Potomac water 48
(E) The retardation phenomenon 51
(F) Isolation of virus from Potomac water 54
Discussion and future consideration 56
Literature cited i

SUMMARY AND RECOMMENDATIONS

(1) The present study was initiated at the request of the United States Army Corps of Engineers, North Atlantic Division, as part of the Northeastern United States Water Supply (NEWS) Study. This study called for testing inclusively 25 strains of human enteric viruses for their resistance to free chlorine in partially treated Potomac water. A portion of the NEWS study is to explore the feasibility of using Potomac river water as an alternative source of supplementary water supply for the metropolitan area of Washington, D.C. after the year 2000. Should the river water be used for such a purpose at that time, viral pollutants would seem to present a potential health hazard to the public. It is hoped that from this study a clarification and/or solution of this problem may be obtained

(2) From reviewing available literature, there is evidence to indicate that drinking waters may carry human viral pollutants and occasionally cause outbreaks of human enteric virus diseases. In recent years these viruses have become increasingly recognized in association with a number of serious human diseases. There is also an indication that the effect of chlorination on viruses has not been thoroughly explored and defined in the past.

(3) This study was completed in July 1971 during which 25 strains of human enteric viruses were completed in testing for chlorine resistance. They were shown to possess a wide range of resistance to the treatment when suspended in Potomac water under identical conditions. As expressed in the number of minutes required for 99.99% devitalization by 0.5 mg/1 of free residual chlorine, the degree of resistance of the viruses are listed below in a descending order: coxsackievirus A type 6 (43.7 minutes); poliovirus type 2 (40.0 minutes); coxsackievirus B type 5 (39.5 minutes); coxsackievirus A type 5 (35.3 minutes); echovirus type 1 (27.0 minutes); poliovirus type 3 (16.7 minutes); coxsackievirus B type 3 (16.2 minutes); poliovirus type 1 (16.2 minutes); echovirus type 12 (14.5 minutes); echovirus type 11 (14.0 minutes); adenovirus type 12 (13.5 minutes); echovirus type 8 (13.0 minutes); adenovirus type 7a (12.5 minutes); echovirus type 9 (12.0 minutes); coxsackievirus B type 1 (8.5 minutes); echovirus type 5 (8.0 minutes); echovirus type 7 (7.1 minutes); coxsackievirus B type 4 (7.0 minutes); coxsackievirus A type 9 (6.8 minutes); coxsackievirus B type 2 (6.5 minutes); adenovirus type 3 (4.8 minutes); reovirus type 2 (4.2 minutes); reovirus type 3 (<4.0 minutes); and reovirus type 1 (2.7 minutes). These findings

are in general agreement with those reported in the literature wherever such information is available. It is clear from these data that the degree of chlorine resistance of any human enteric virus is unpredictable.

(4) From a limited comparative study, the treated Potomac water was found not to be significantly different from the demand-free water when used as the suspending medium for virus. The efficiency of chlorination on viruses is directly related to water temperature, dosage of free chlorine and length of contact time. These findings also corroborate those described in the literature.

(5) Several of the viruses tested were found to show an obvious retardation of the initial rate in the later part of inactivation. The reason for this observation has not been determined at this time. Theoretically, this could be explained by the presence of viral aggregates in preparations used in these experiments. The number of aggregates present was calculated and appears to be very small. In all instances, the aggregates constituted <0.1% of the total viral population. The aggregates would theoretically simulate more closely viruses occurring in natural environment. Indication is that the naturally occurring virus may be much more resistant to the chlorination process than virus seed that was prepared and used for experimentation in the laboratory.

(6) Based on the data from this study, no definite pattern can be established for chlorine resistance of all the human enteric viruses. In addition, little is known of the resistance exhibited by the naturally occurring forms of virus. Without such information, valid guidelines cannot be developed for the chlorination process in water treatment to specifically safeguard water supplies from virus hazards. Thus, it is recommended that this study be expanded to ascertain the comparative chlorine resistance of all known types (100+) of human enteric viruses in both purified and natural forms and those factors in the environment which may affect its efficiency.

INTRODUCTION

Until the beginning of this century, human enteric bacteria continued to cause a significant number of diseases and deaths in man throughout the world. This trend was drastically changed during the ensuing 3-4 decades by implementation of modern water supplies and their separation from sewage disposal systems. These measures almost eradicated these diseases in Europe and North America. In recent years, however, there has been a growing concern of the possibility that modern water supplies, even in this country, may be polluted with low levels of human enteric viruses and serve as a vehicle to transmit these viruses to man. To date, there have been 53 outbreaks of infectious hepatitis and 8 outbreaks of poliomyelitis reported as waterborne in the literature. Although no sporadic cases of viral diseases have been described as waterborne, epidemiologic patterns indicate that a certain number of the index cases of infectious hepatitis may be associated with drinking water. If this were true, transmission of other types of human enteric viruses by the water route would appear possible.

According to a recent assessment made by Chang (1), the water treatments used today can probably reduce the viral pollutants in

finished waters to a minimum level, if these treatments were carried out properly and if no accidental contamination of raw water supplies occurred. He concluded that the most reliable step of water treatment for elimination of viral pollutants is probably the application of a suitable disinfectant. Chlorination has been used in this country for approximately half a century and its effectiveness against enteric bacteria has been well demonstrated in the laboratory as well as in the field. As for its effectiveness against the human enteric viruses, however, there is still insufficient data to conclude that the numerous types of virus known today and the many forms of viruses which may exist in nature, could be completely eliminated by the average water treatment process. In the past two decades, reports from several laboratories described the effect of chlorination against 9 strains of virus in water. The viruses tested were derived from various tissue culture and animal systems and subjected to different processes of manipulation. On most occasions, the experimental assays used insufficient number of tissue cultures or laboratory animals. Based on this kind of data a direct comparison of the relative resistance of these viruses to chlorine is virtually impossible. This information is also inadequate for field application for the following reasons:

(a) The number of viruses tested was too small to constitute a fair sampling of the known viruses thus generalization

of the findings are not without pitfalls.

(b) The virus preparations used for these studies were obtained from and tested in laboratory systems. The responses of viruses so derived cannot be translated directly into the responses of those occurring in the natural environment.

(c) The effect of many environmental factors which may influence the disinfective efficiency of chlorine, has not been thoroughly explored and defined. One may encounter unexpected and unexplainable difficulties if he attempts to apply this information to the more complex and rapidly changing field situations.

As estimated by the Army Corps of Engineers, the Department of Defense, the water of the Potomac river estuary should be considered at least as one of the alternatives for supplementing the present sources in the Washington metropolitan area beyond the year 2000, if the population continues to increase at the present rate. One of the major concerns over such a plan is how effective is the chlorination process in eliminating the many viral pathogens which may pollute the raw water supplies. The objective of this study is to determine the difference, if any, in resistance to chlorination of 25 strains of human enteric virus suspended in the water from the Potomac river estuary.

It was hoped that from this study sufficient data might be accumulated to formulate appropriate guidelines for chlorination of water supplies. By July of this year the whole study has been completed as originally planned. This report summarizes all the data obtained from the laboratory; discussed their significance in relation to the initial objective and presents pertinent future considerstion.

BACKGROUND

Literature pertaining to the following three aspects of 'water virology' is briefly reviewed:

(A) the potential health hazards from viruses in water;

(B) the epidemiology of waterborne virus diseases; and

(C) the efficiency of water treatments in elimination of viruses.

(A) The potential health hazards from viruses in water:

Any virus, excreted in feces or urine and capable of infecting man when ingested, could be transmitted by water. From a practical viewpoint, only those viruses that are discharged in large numbers in feces and have a long survival in the water environment, need to be concerned with. The recognized groups of human viruses fulfilling these criteria include: the enteroviruses, having the subgroups of polioviruses, coxsackieviruses, and echoviruses; the virus(es) of infectious hepatitis (IH); the adenoviruses; and the reoviruses. As shown in Table 1, the total number of virus types known today that compose these groups is around 100 excluding the IH agent(s). The latter agent(s) has not been positively isolated or identified by any laboratory procedure to date. Its presence in sewage and water is only evidenced by outbreaks directly associated with drinking water and indirectly associated with raw

shellfish and by infection of human volunteers with polluted water.

Viruses of animal origin also deserve consideration. Those that can find their way into waterways could be transmitted to man through supply systems or recreational facilities. Examples of these viruses are enteric viruses of domestic animals; those from fish and other aquatic animals and fowl and many others. There is no need for immediate concern of these "foreign" viruses today. A constant vigilance, however, should be maintained on any new development which may have implications to human health.

The various sources of viral pollutants and possible routes of their transmission through water to man are illustrated in Figure 1. As shown, there are many pathways through which viruses can contaminate water, as there are many opportunities for them to infect man. Thus, the virus can continually perpetuate in the "host-to-susceptible" cycle with water as one of its transport vehicles. It is unfortunate to note that the ecology and natural history of human enteric viruses is poorly understood today.

As is generally known, a very small number of virus particles constitutes an infective dose for a susceptible host. The data reported by Plotkin & Katz (2) are presented in Table 2 and clearly illustrate this point. Therefore a water supply containing even a very low concentration of virus, could result in widespread sporadic

cases in a community. At higher viral concentrations, which usually result from accidental contamination, epidemics are precipitated. In recent years, a total of 53 waterborne outbreaks of hepatitis have been reported in the literature. The low incidence of recognized diseases associated with other types of human enteric viruses may be explained, at least in part, on the basis of the very low ratio of the number of clinical cases to the number of subclinical cases, e.g. one of 100-1000 infections of a coxsackievirus or echovirus is actually ill. Thus, there could be an outbreak of a given viral infection in a community, the great majority of which are of subclinical nature and therefore go unnoticed. The cases with overt clinical symptoms appear so sporadic that the epidemic nature is unrecognized and these cases are usually attributed to the common person-to-person spread. Thus, it appears possible that the sporadic incidence of enteric virus diseases, such as infectious hepatitis, in this country, could have come at least in part from a low level pollution of water with these viruses.

The clinical and epidemiologic experience accumulated in recent years permits development of the following two concepts on pathogenicity of human enteric viruses:

(1) Clinical illnesses:

One or two decades ago, clinicians and virologists generally

believed that the various groups of human enteric viruses caused only self-limiting diseases in man except for the poliovirus. The diseases associated with these viruses as described in early literature are listed in Table 3. In the past decade, however, an increasing number of serious diseases have been associated with some of these viruses. A partial list of such diseases is given in Table 4. As shown in this table, these diseases by no means are self-limiting. For instance, the heart damages caused by coxsackieviruses may impair the patients' activity for many years and finally claim their lives. The acute interstitial myocarditis associated with echoviruses could result in deaths of patients in a matter of days. The insulin-dependent diabetes connected with coxsackievirus infection may disable patients for decades. Even more distressing is the fact that coxsackievirus infections occur mostly in children and infants who may suffer from the residual organic lesions for many ensuing years.

(2) Subclinical infections:

Subclinical infections of enteric viruses in man are far from innocuous to health; also, certain enteric viruses are associated with serious delayed effects on the infected

persons or even their offspring. Deserving particular attention are the coxsackieviruses which have been incriminated as teratogenic agents. The affinity of these viruses to heart tissue has become increasingly recognized from experimental studies as well as clinical and epidemiologic observations. Brown and Evans (3) reported high incidence of congenital heart anomalies associated with the infection of mothers with type B coxsackievirus during the first trimester of pregnancy. About half of the infected cases were subclinical and could only be detected by serologic examination. A portion of individuals subclinically infected with the same group of virus developed insulin-dependent diabetes (4). In addition, the role of the enteric virus etiology of oncogenesis in man has as yet to be ruled out (5).

In conclusion, there are many types of virus which can pollute waterways and subsequently contaminate the water supplies, thus leading into the water intake for human consumption. Of immediate concern are those from the human enteric tracts. Viruses of non-human origin, although they present no known health hazard today, should be observed for possible new developments on health implication in the development of disease. There is a growing number of

serious diseases which have been associated with human enteric viruses in recent decades. The list of such diseases will undoubtedly expand with time. To produce an infection with a given virus in a susceptible host only requires a few viral particles. The detrimental effects associated with these viruses, demand that we look at all possible modes of their transmission.

(B) Epidemiology of waterborne viral diseases:

While modern well-operated water systems have eradicated the non-food borne enteric bacterial diseases, there has been an increasing awareness of waterborne enteric viral diseases in recent years. Bodian & Horstman reported, before the emergence of poliovaccines, that the poliomyelitis incidence definitely increased as the enteric bacterial diseases were phasing out. Basically the mode of transmission for the two groups of enteric microorganisms is the same, i.e. oral-anal route. Eradication of the bacterial diseases has been ascribed to improved water supply and sewage disposal. Epidemiologists, however, contend that the incidence of non-epidemic enteric virus diseases is an outcome of only person-to-person contact. This contention appears to be of intuitive nature and one would wonder why the incidence of bacterial diseases, such as typhoid fever, which shares the same mode of transmission, has shown such a dramatic decline.

A total of 53 waterborne outbreaks of infectious hepatitis were reported in the literature. The pertinent information on these outbreaks is summarized in Table 5. During the same period, at least until 1957 when poliovaccine became available, only 8 waterborne outbreaks of poliomyelitis were reported. The pertinent information of 6 outbreaks is summarized in Table 6. The epidemiologic data indicate that viral diseases can indeed be transmitted by drinking water. Questions may be raised as to why there were more outbreaks of hepatitis than poliomyelitis. At least two explanations may be given at present. (a) The hepatitis virus is probably more hardy than the poliovirus, thus surviving longer in the aquatic environment and escaping the effect of various water treatments. (b) From analysis of existing data, the average attack rate of hepatitis virus in waterborne outbreaks appears much higher than that of poliovirus, i.e. 15% versus 0.2%. Thus, the former outbreaks were readily identifiable as transmitted by a common vehicle, whereas the latter might have been missed as such and/or considered to have been spread by other modes. It is conceivable that other enteric viruses transmitted by water may behave in a similar manner to poliovirus; such outbreaks could have been overlooked in the past.

The "non-bacterial" gastroenteritis is a well-recognized clinical entity today. Many epidemics of this disease have been intuitively

ascribed to viruses, although very few have been thoroughly studied for the causative agents. Experimental work by Gordon, Ingram and Korns (6) and Jordan, Gordon & Dorrance (7) indicates that some of the gastroenteritis or diarrheal diseases are caused by certain types of human enterovirus. Lobel, Bisno, Goldfield & Prier (8) suggested that viral diarrhea may be transmitted through the water route, then followed by a secondary person-to-person spread. The role of water in transmission of diarrheal diseases of different etiologic agents is confirmed by the report of Weibel and co-workers (9). They listed 142 epidemics of diarrheal diseases that occurred in this country from 1946 to 1960 affecting more than 18,000 persons. Mosley (10) believes that a significant portion of the number could have been caused by viruses. Furthermore, the number given above is probably very conservative, since most cases were not reported by the physicians, who were probably consulted only by a small fraction of cases.

The various possible modes of transmitting human enteric viruses, in general, are depicted in Figure 2. As clearly shown, the more direct routes of spread for these viruses have been well documented in literature. As illustrated in the shaded area, the longer and more indirect routes of transmission are poorly understood today. The pathogen concentration in the conveying vehicles, such as water,

is bound to be very low under normal circumstances and such infections are highly sporadic and irregular. The conventional epidemiologic tools are not sensitive enough to establish the disease-source relationship. Also, it is clear that a different and quantitative laboratory-epidemiologic approach is required in such disease-source investigation in order to understand the behavior of non-epidemic enteric diseases. Such an understanding is essential to the development of effective control measures in the future.

It is clear from the above information that the occurrence of waterborne outbreaks of infectious hepatitis has been well documented in the literature. There is a strong suspicion that outbreaks of poliomyelitis and viral gastroenteritis have been associated with polluted water supplies. If a virus infection has a short and uniform incubation period and produces a characteristic overt disease with relative frequency, carriage of the virus by water routes can be traced with a fair degree of accuracy. The hepatitis agent(s) seem to fulfill all above criteria except the short incubation period. The lack of evidence for waterborne incidence of other enteric viruses, however, could be attributable to the variable nature of illness produced: (a) these viruses produce disease in only a small fraction of those who become infected; (b) they produce diseases with widely variable incubation periods and clinical symptoms; and (c) they are also readily spread by direct human contact.

Even though water transmission of viruses is very difficult to trace in many instances, the presence of these pollutants in sewage effluents, streams and other waters cannot be viewed with equanimity.

(C) Efficiency of water treatments on elimination of virus:

There is scant information from field studies concerning the efficacy of water treatments on elimination of human viral pathogens. Such data will not be forthcoming until a suitable method(s) becomes available which can be used to quantitatively detect the small numbers of virus in large volumes of waters. It is apparent that examination for virus in both raw and finished water of municipal supplies is the most direct and desirable way to obtain factual information on efficiencies of the water treatment processes as well as on the role of water in transmission of viruses.

On the other hand, a fair amount of information from laboratory studies is available concerning efficiencies of these treatment processes in removal and destruction of virus. These studies were conducted under controlled conditions and the evaluation of all processes was carefully and comprehensively carried out. At this point, it should be stressed, however, that the number of viruses studied is very small, thus, one should be cautioned against generalization of these data. For instance, the removal and destruction of

the most important virus, the infectious hepatitis agent, in water, has been studied in a very limited manner and by the sole use of human volunteers.

The following summarizes the available information on the efficiency of each process on viral removal:

(a) Storage:

Undoubtedly there are many factors which may affect virus survival in water. The data on survival times of several viruses at different temperatures reported by Clarke et al (11) are insufficient to calculate the comparative survival rates of viruses and the temperature coefficient for predicting virus reduction in storage reservoirs. In general, a 99.9% reduction takes place in a few weeks in warm months and in a few months in cold months. There is presumptive evidence to indicate that infectious hepatitis virus survived ten weeks in well water stored in a laboratory (12). Prolongation of the incubation period of the disease, however, was observed in human volunteers fed with the stored samples. This seems to denote that a reduction in viral concentration might have occurred during that period of storage.

A study of virus survival in estuarine water is being

conducted by Akin and Hill (13) in the Water Supply Programs Division's Gulf Coast Laboratory, Their data essentially shows that the survival of different enteric viruses is highly unpredictable. This can, at least in part, be attributable to a number of undetermined factors and trace elements existing in water of various sources.

The pH of most surface water lies within the range well tolerated by the human enteric viruses tested so far. Salts sometimes may have some adverse effect, but may exert protective effects on other occasions (14). Long storage is effective in reducing viral concentrations in reservoirs. Summer temperature and long periods of sunshine should facilitate their reduction. There is, however, no safeguard against short circuits caused by convection, wind, or rain, which may shorten the travel of virus through the reservoir to a matter of hours instead of weeks.

Thus, it may be concluded from the above information that the storage practice normally employed in water treatment is not to be depended upon as an effective step for elimination of enteric virus content in raw water, but it may reduce their numbers to some extent under normal circumstances.

(b) Flocculation and sedimentation:

Laboratory data (15) and data from a pilot plant study (16) indicate that a 99% removal of viruses can be achieved, if good floc formation is assured. A properly carried-out process is important to achieve a satisfactory result. The factors assuring success include adequate coagulant concentration, absence of interferring substances, proper pH control, thorough mixing, and the like. Flocculation may be considered as one of the effective means for removing viruses from water supplies, however, the many conditions required to reproduce adequate flocs are demanding. Adequately formed flocs for good virus removal in water treatment plants is probably an ideal rather than routine.

Chang et al (17) showed that the flocculation process is analogous to the step of colloidal chemical reactions involved in serological agglutination or precipitation, i.e. the formation of metal cation-virus complexes which aggregate further to form visable and suitable masses. It was shown that once the virus $Al(OH)_3$ complex is formed, the reversal is drastically reduced.

It may be concluded from the above data that this

process is effective in reducing virus concentration and the total content of organic matters in water. Thus, chlorination or other chemical disinfection process becomes more effective in finally destroying the viruses. Flocculation should also be considered a prerequisite process for disinfection.

(c) Filtration:

Filtration was first applied in 1829 to purify the Thames River water of London, used as a source of the metropolitan water supply. This process is not to be depended on for any significant removal of viruses in water. Most of the earlier workers obtained poor results in laboratory studies.

The more quantitative study of Robeck et al (16) showed that 1-50% of poliovirus was removed by rapid sand filtration at rates of 1880-8640 US gal/d/ft^2 (about 120,000-350,000 1/d/m^2) and 22-90% by slow sand filtration at a rate of 50 US gal/d/ft^2 (about 2000 1/d/m^2). Rapid sand filtration, combined with chemical flocculation is, however, effective in removing bacteria and viruses from water. Since the above study used only one strain of poliovirus, it remains to be seen whether or not these results are applicable to other types of virus.

Factors which may affect the efficiency of sand filtration include: original turbidity; success of flocculation; types of sand used; rate of filtration; presence of organic matters; pH of the water; the types of coagulant-aid used; etc. The efficiency of virus-removal, in general, appears to be higher when the process of flocculation and sand filtration are carried out together. For instance, Robeck et al (16) obtained a better than 99.7% removal of the type 1 poliovirus by the combined procedure, whereas the total removal was much less when the two processes were carried out independently.

Observations of the infectious hepatitis agent by Neefe et al (18) indicate that an insignificant removal of this virus was achieved by diatomaceous earth filtration. This was evidenced by the fact that the incubation periods in human volunteers infected with the flocculated and filtered water were not significantly lengthened as compared to those in persons infected with the not filtered water.

All data for the study concerning infectious hepatitis virus should not be considered conclusive because insufficient number of volunteers were used and the sensitivity and accuracy of this method in quantitation of the virus

is not well defined to date.

(d) Disinfection:

The final and most dependable step in water supply treatment practice is the application of a suitable disinfectant. In this country chlorination is generally preferred to any other process. The objective of disinfection is to safeguard the consumers against water-borne infectious diseases. The basic requirements for disinfectants suitable for this purpose are:

(1) No acute or chronic toxic effect on human and other living organisms consuming the water over long periods of time.

(2) Effective in killing all biologic pollutants at low concentrations.

(3) Inexpensive and convenient for mass application.

Chlorine, especially HOCl, was found to fulfill all the above requirements. Chlorination of drinking water was first practiced in England in 1880. The use of chlorine compounds has met with considerable success in this country since the beginning of this century. Some other compounds have been studied and proposed as

substitutes, but none of these has gained wide acceptance.

(i) Chlorination:

When gaseous Cl_2 is dissolved in water, the Cl_2 hydrolyses into HOCl and HCl. The HOCl dissociates into H+ and OCl^- ions. HOCl is far more powerful than OCl ion in devitalizing microorganisms. Below pH 6.0, practically all titratable Cl exists as HOCl; above pH 9.0, nearly all Cl exists as OCl^- ion. Within this pH range, varying amounts of HOCl exist governed by the dissociation constant. Chloramines are formed when HOCl reacts with ammonia or amino compounds in water. The titratable chlorine in these N-chlor compounds is known as "combined chlorine". Their virucidal efficiency is much inferior to HOCl known as "free chlorine". Kelly and Sanderson (19,20) showed that the efficiency of HOCl in devitalizing human enteroviruses is more than 50 times greater than that of the ammonia chloramines. Organic chloramines, e.g. chloramine-T, are even less effective. The limited data on hepatitis virus reported by Neefe et al also indicate that free chlorine is more active than the combined forms. Human enteric viruses

differ in their resistance to free chlorine. Some of the more quantitative results on bacteria and less quantitative data on 9 viruses reported in literature are summarized in Table 7. Adenovirus type 3 was the least resistant among those tested; it seems even less resistant than E. coli. Poliovirus type 1 and coxsackievirus types A2 and A9 appear to be most hardy among the tested viruses. Some of the differences may be attributable to the methods used in assaying the surviving viruses. The resistance of hepatitis virus appears quite high from the limited studies using human volunteers. Since this virus has not lent itself to laboratory manipulation and other enteric viruses were tested under varying conditions, a direct comparison of their resistance to chlorine at this time is at best an approximation.

Since disinfection is a "rate process", it is difficult to destroy all the pathogens in water supply by such a process. For instance, even if a chlorination process can facilitate a 99.9999% destruction of virus, a 1×10^8 gallon supply system

containing one virus unit/gallon would still carry 100 infective virus units in a day's supply. A valid guideline for water treatment with a known safety margin cannot be established until the levels of viral pollution of raw and finished water and the relative resistance of all enteric viruses to chlorine are defined.

(ii) Iodination:

Chang (21) reported that iodination can be conveniently used to disinfect small supplies where pretreatment and controlled chlorination are impractical. The virucidal efficiency of I_2 is about 1/200, and that of HOI is about 1/5 the efficiency of HOCl (22). A 99.9% destruction of both viruses and amoebic cysts can be achieved, if long contact times are used. The amounts of iodine used to treat water should not exceed 4 ppm. When the water contains relatively low concentrations of organic matter, a 99.9% destruction of enteric viruses can be attained by using 0.6 - 1.0 ppm iodine in 30 minutes of contact. Thus, this would limit the raw source to relatively clean water which should not be more than

slightly polluted.

(iii) Ozonation:

Coin (23) reported that 0.4 ppm of ozone can destroy poliovirus type 1 in tap water in 30 minutes and less ozone is required to inactivate poliovirus types 2 and 3. Trahtman (24) obtained a 99.9% destruction of enteroviruses with 0.2 ppm of ozone, but no contact time was mentioned. Their method(s) of determining residual ozone was not fully explained, hence, these residuals could have contained H_2O_2 which is usually formed in ozonated water. This treatment has the advantage of not introducing odor and taste problems and the disadvantage of difficulty in dispersing it uniformly and in maintaining residuals in water for any appreciable length of time. It, however, is useful in pretreatment of water prior to chlorination to reduce the dose of chlorine required and the pathogen load. Thus, when used as a preliminary step this process would render the chlorine treatment more effective and less objectionable.

(iv) Radiation:

Both UV and gamma radiation are potential means of

water disinfection. A large amount of information is available on the effect of UV irradiation on inactivating human enteric viruses. Huff et al (25) described a UV system designed to disinfect small amounts of water in ships. Their data indicate that this device could satisfactorily inactivate all types of poliovirus, echovirus type 7 and coxsackievirus type A9 at concentrations of 10^2 - 10^4 PFUs/ml. The efficiency of this treatment is influenced by turbidity and color of water, presence of iron salts and organic compounds, etc. The authors also showed that the virucidal efficiency was significantly reduced when 9 units of "instant tea" color were added to the water. Hill et al (26) reported that a 99.9% reduction of 7 strains of human enteric viruses was obtained in 20-30 seconds by UV radiation when the viruses were suspended in filtered seawater from the Gulf Coast region.

Sullivan and associates (27) described the virucidal effect of gamma rays from a cobalt-60 source. Because of its greater penetrability, gamma rays are less affected by outside factors than UV rays.

A 99.9% reduction was achieved for five enteric viruses by gamma radiation at a dosage of 1.2 megarads. At this time, the initial cost for using gamma radiation of municipal water supplies would be prohibitive.

Conclusions and comments

The data accumulated in the past two decades on efficiency of water treatment processes in removal and inactivation of viruses have been reviewed. Conclusions which may be drawn from these studies follow:

(1) Some of the basic mechanisms and the relative efficiency of the water treatment processes in removal and destruction of enteric viruses have been elucidated.

(2) The number of strains studied is very small and does not permit the establishment of a general pattern for all types and groups of human enteric viruses known today.

(3) Little is known today about the virus density of various surface waters. Thus, it is impossible to accurately assess what safety margin the present treatments may provide when polluted sources are used.

(4) The occurrence of waterborne hepatitis outbreaks indicates that the potential danger of such episodes always exist and outbreaks will recur, if the conditions of the raw water and the treatment practices are altered in favor of the virus population.

(5) There is also the possibility of low level transmission of virus by water. This may be responsible for at least a portion of the annual incidence of infectious hepatitis and other enteric virus diseases.

From the foregoing conclusions it is felt that a quantitative laboratory-epidemiological investigation is urgently needed in order to determine the true situation with regards to waterborne virus diseases.

MATERIALS AND METHODS

Viruses: The viruses included in this study are listed in Table 8. All the seed viruses were obtained from the Reference Reagents Branch, NIAID, NIsH, Bethesda, Maryland. Before use in chlorination experiments, these viruses were serially passaged in appropriate culture systems, as indicated in this table, until adequate titers were obtained. All stock viruses thus prepared were assayed for titer by appropriate test and identified again by using the standard monovalent specific antiserum, obtained from the Reference Reagents Branch. All the stock viruses were subjected to the following treatments to minimize their chlorine demands and reduce viral aggregates:

(a) The stock virus was frozen and thawed 3x in situ before harvested and pooled.

(b) The pool of virus was centrifuged at 2000 rpm for 30 minutes.

(c) The supernate was dialyzed consecutively in demand-free water 3 times at $4^{o}C$.

(d) The dialysate was centrifuged at 10,000 rpm for 1 hour or filtered through a 0.22 Millipore membrane.

(e) Following the centrifugation, the upper half of the virus

in centrifuge tubes was withdrawn carefully.

(f) Both the filtrates from the step (d) and the supernates from the step (e) were checked for chlorine demand at a ml/liter of demand-free water. Aliquots of those with minimum or no demand were distributed into test tubes and stored at -20°C until ready for chlorination experiments.

Demand-free water: The demand-free water used throughout this study was obtained from a glass still after double distillation. Before use in experiments, the chlorine demand of each batch of water was determined by addition of 1 mg/1 of HOCl and checked periodically for free residual chlorine for a period of 30-60 minutes. The water used for all experiments presented in this report was free of chlorine demand.

Potomac River estuarine water: A sample of 200 gallons of the Potomac River estuarine water used throughout the experiments was collected from the Potomac River estuary about one mile below the sewage discharge from the Washington, D.C. Sewage Treatment Plant on February 11, 1970. This sample represents a typical composite of the river estuarine water supply at that time. It was coagulated by an appropriate amount of Al hydroxide in a pilot tank mixer at the Washington, D.C. Water Treatment Plant. After this, the water was filtered through a pilot sand-filter at a slow rate. This water was then dis-

tributed into a number of sterile 50-gallon drums which were transported back to this laboratory by truck. Upon arrival, all water from these drums were pooled into a steam-cleaned 300-gallon plastic tank and thoroughly mixed by stirring with sterile wooden paddles. The water in the jugs was shaken thoroughly before use for each experiment. It was also characterized periodically to determine whether or not there was any change of a chemical nature occurring during storage.

Glassware: All glassware and pipettes were first acid-washed and finally rinsed with chlorine demand-free water. By repeated checking none of the glassware used in this study showed detectable chlorine demand.

Chlorination experiment: The protocol for testing the first 6 representative viruses is summarized in Table 9. The objectives of these experiments are:

(a) To compare the Potomac and demand-free water for their suitability as the medium in suspending the viruses for chlorine resistance determination.

(b) To confirm the temperature effect on virucidal potency of free chlorine in water.

(c) To determine virucidal efficiency of free chlorine in relation to dosages and contact time.

After completion of the testing of the 6 viruses, it was agreed by the engineers of the Corps of Engineers and the virologists in the Northeastern laboratory that all remaining viruses be tested under the following conditions:

1. Use the Potomac water as virus suspending medium.
2. Use a single level of chlorine, i.e. 0.5 mg/l.
3. Carry out all tests at one temperature, 2°C and one pH, 7.8*.
4. Sample more often in the first period of 30 minutes of chlorination, i.e. at 0, 2.5, 5.0, 7.5, 10, 15, 20 and 30 minutes of contact time.

All samples collected from these experiments were distributed into 5 ml aliquots and stored at -20°C until assayed.

Viral assays: All samples were assayed at least twice: (a) The preliminary assay of each sample was performed by using a small number of tissue culture bottles, tubes or animals and several dilutions judging by the titer of the stock virus; (b) the final assay of each sample was determined by using triplicate dilutions each in 2-4 tissue culture bottles or triplicate dilutions each in 5 tissue culture tubes and only one appropriate dilution as determined by the preliminary assay.

Plaquing: A number of enteroviruses were plaqued in 3 oz. prescription bottles containing monolayer of primary African green monkey kidney tissue culture cells. The techniques for

* pH 7.8 was chosen because the Washington water was at this pH before chlorine was added.

preparing the tissue culture bottles and later plaquing were essentially those described by Hsiung and Henderson (28). All reoviruses were assayed in 3 oz. bottles containing a monolayer of L929 strain of mouse fibroblast tissue culture cells. The medium used was essentially the same as that for monkey cells except that 0.5% of pancreatin was added to the overlaying agar medium.

$\underline{TCD_{50}}$ titration: All adenovirus samples were titrated by this method, using primary human embryonic kidney (HEK) roller tubes from the Flow Laboratories, Incorporated. A triplicate of serial 10-fold dilutions were made in nutrient broth tubes for each sample. An inoculum of 0.1 - 0.2 ml was delivered into each tube, followed by addition of 0.8 - 0.9 ml of maintenance medium. Five tubes were used for each of the triplicate dilutions. Cytopathic changes of the culture cells were observed under the microscope and recorded for a period of 3 weeks or longer. During this period, the cultures were fed with fresh maintenance medium every 2-3 days. The TCD_{50} titers were calculated by the formula of Reed & Muench (29) and are expressed as $TCD_{50s/ml}$ throughout this report.

$\underline{LD_{50}}$: Certain A types of coxsackievirus were titrated in suckling mice (1-3 days old). A series of 10-fold dilutions were

made in buffered saline for each sample. Two tenths ml was inoculated intraperitoneally into each suckling mouse and one litter was used for each dilution. The mortalities of the inoculated mice were observed for 2 weeks and the LD_{50} titers were calculated by the formula of Reed & Muench. Each sample was tested three times in this manner. Throughout this report, the LD_{50} titer is expressed as the number of LD_{50}s/ml.

Chlorination: The water samples were chlorinated with a diluted stock solution of calcium or sodium hypochlorite. The stock solutions contained 7 mg/ml of chlorine. Immediately before each experiment, the stock solution was diluted to contain 0.5 mg/ml of chlorine. An appropriate volume of this diluted stock was added to water samples to achieve the desired dose levels. Both the stock and diluted solutions were calibrated by using the Fischer-Porter Titrator, Model 17T1010. For the experiments using demand-free water, chlorine solution was added immediately after addition of the tested virus. For the experiments using Potomac water, the chlorine solution was added approximately 2 hours before the virus. At that time, the residual-free chlorine was stabilized at the desired level. In general, 1.5 mg of chlorine was required to dose a liter of the Potomac water in order to achieve a level of 0.5 mg/l of free chlorine 2 hours later.

Chemical assays:

Sulfates: To a 100 ml sample of water add 5.0 ml of glycerol-

alcohol conditioning solution. Stir and add barium chloride crystals. Transfer solution after 1 minute stirring to a suitable photometer (Hach turbidimeter) and record maximum reading. Compare results to a standard curve constructed by using known sulfate concentration 0-40 mg/l (30).

Orthophosphate: The following procedure was used. Construct a calibration curve using known concentrations of 0.05 - 1.0 mg phosphate. Place 35 ml of standard and unknown solutions in 50 ml flasks. To each add 10 ml of vanadate-molybdate reagent and dilute to volume. After 10 minutes, measure the solution in a spectrophotometer at 400 mμ, 440 mμ, 470 mμ, and 490 mμ, and construct a curve for each wavelength. Read the unknown off the appropriate curve.

Nitrates: Nitrates in water were analyzed by the Modified Brucine Method. Construct a standard curve for nitrate using 10 ml aliquots containing 0-10 ug nitrate nitrogen. To the standard and unknown solutions in a test tube, add 2 ml 30% NaCl, 10 ml sulfuric acid, and 0.5 ml brucine-sulfanilic reagent. Immerse in a 95^{o}C water bath for 20 minutes. Cool and read yellow color in a spectrophotometer at 410 mμ. Read unknown concentration off the standard curve.

Chlorine: The standard method was used. Water samples were

titrated with standard phenylarsine oxide using a Fischer-Porter Titrator Model 17T1010. The concentration of chlorine in ppm or mg/l was read directly off the burette in the instrument (30).

Chloride: Chloride concentrations of water were measured by use of the specific (chloride) ion electrode. Construct a standard curve measuring chloride solutions from 10 to 10,000 ppm versus the MV potential readings on the Beckman Expandomatic pH Meter. Utilize the Beckman Chloride Probe in conjunction with the Reference Electrode (Calomel). Measure the MV for unknown solutions and read concentrations from the standard curve (32).

Sodium: Water analyzed was measured for sodium together with standards required for calibration curve using atomic absorption spectrophotometry. The instrument used was the Perkin-Elmer 303 AAS using the sodium arc discharge lamp (33).

pH: The standard method was used. Measure the pH of water using a Beckman SS-2 pH Meter, Glass Electrode vs Reference (S.C.E.) Electrode (30).

Alkalinity: The standard method was used. Titrate an aliquot of the water with 0.02N standard sulfuric acid using phenolphthalein indicator to determine the phenolphthalein alkalinity.

Add methyl orange indicator and continue titration to the methyl orange endpoint to give total alkalinity (30).

Turbidity: Water turbidity was measured by use of the Hach Chemical Company Laboratory turbidimeter Model 2100. The water samples were tested in the calibrated Hach Instrument according to their instructions. The result was read directly in Jackson Turbidity Units (34).

Hardness: A modified standard method was used for this assay. Measure calcium and magnesium contents of the water with Perkin-Elmer AAS and appropriate lamps. Derive hardness by calculation. Ca x 2.497 + Mg x 4.116 to convert to total hardness in ppm as expressed in $CaCO_3$ equivalents (30).

Total dissolved solids: A modified standard method was used. Evaporate a filtered 100 ml water sample in a 150 ml beaker. Initially evaporate on a hot plate and later transfer to a drying oven. Cool to constant weight. Note difference between tared beaker and residue (30).

EXPERIMENTAL RESULTS

(A) Characterization of the Potomac water:

A typical composite of the Potomac river estuary water was collected, treated, pooled and stored at 4°C, as described in the previous section. Before use for each experiment, the water was thoroughly shaken and poured into a sterile container which was carried to the virus laboratory immediately. During the period of storage, the water was repeatedly characterized by the various tests. It was hoped that these tests would detect the change, if any, of the water which might occur during the long storage. The testing results on the water performed in February and August, 1970 and May, 1971 are summarized in Table 10. It is clear from these data that no apparent change of a chemical nature occurred during the entire period of study.

(B) Determination of experimental parameters:

Six representative viruses were used to evaluate the essential parameters needed for testing all viruses as listed in Table 8. The 6 viruses were: poliovirus type 1; coxsackievirus types A9 and B1; echovirus type 7; adenovirus type 3 and reovirus type 3. The procedures employed to test each virus have been described. These experiments were conducted in an attempt to

answer the following questions:

(a) Was the Potomac estuarine water a suitable suspending medium for conducting these tests?

(b) What was the effect of temperature on the virucidal efficiency of chlorination? What was the choice of temperature for later experiments, if one temperature was to be used?

(c) What was the optimal dose of free chlorine to be used in these experiments?

(d) What were the adequate time intervals for sampling during the experiments?

The experimental results for the poliovirus type 1 are summarized in Tables 11-13; those for the coxsackievirus type A9, in Tables 14-16; those for the coxsackievirus B1, in Tables 17-19; those for the echovirus type 7, in Tables 20-22; those for the adenovirus type 3, in Tables 23-25; and those for the reovirus type 3, in Tables 26-28.

As shown in Tables 13, 16, 19, 22, 25 and 28, the residual chlorine applied to waters in these experiments was kept well within the wanted levels with only a few exceptions. In general, addition of small amounts of the chlorine solution was necessary to bring the free chlorine up to the desired level in the early

part of the experiments. This indicates that the stock virus added to water probably contains some chlorine demand which might be attributable to the virions *per se* and/or some contaminating organic matters in the virus preparations.

The assay results for the viruses are given in the remaining tables. It is clear from these data that the 24 sets of virus controls are all within the limits of assay variations. As for the devitalizing rates of these viruses under varying experimental conditions, all viruses were killed rapidly by free chlorine within a short period of contact. These results in general corroborate those reported in the literature. The reovirus, however, had never been studied in any other laboratory and was shown to be extremely sensitive to the disinfecting effect of chlorine. This observation is of particular interest in that the size of virus has apparently played no role in the degree of chlorine resistance. This assumption is further supported by the experimental results presented in later sections.

Based upon the individual parameters studied, these data were regrouped, tabulated and further analyzed. The data

illustrating the effect of temperature on virucidal potency of chlorine are presented in Table 29. The positive numbers in the difference columns denote that a higher potency was observed at a higher temperature, whereas the negative numbers denote the reverse. In this table there are 12 positive numbers and only one negative number. Thus, one may safely conclude that chlorination is definitely more efficient in devitalizing viruses at the higher temperature. Since only two temperatures were used in these experiments, the data were insufficient for calculation of temperature coefficient.

These data were analyzed to detect the influence, if any, of the suspending media used, the demand-free and Potomac water, on the disinfecting potency of chlorine on virus. The pertinent results are summarized in Table 30. As shown in the difference columns there are 11 positive numbers and only one negative number. The positive numbers indicate that the chlorine was more efficacious in the demand-free water than in the Potomac water. The negative number means the reverse. Thus, it is clear from this comparison that the Potomac water had a slight deleterious effect on chlorine in inactivating viruses. Since the action of chlorination was considerably slowed down at $2^{o}C$, the values obtained at this temperature are probably more valid than those obtained at $22^{o}C$.

The relation of the concentration of chlorine to the disinfecting activity is illustrated in Table 31. As shown in the difference columns, a total of 13 differences are positive numbers. The one negative value was unexpected and could be attributable to some experimental error either in manipulation or in final assays. Thus, it is clear from this analysis that the more free chlorine was there in water, the greater was the virucidal activity.

In summary, from this preliminary study on 6 representative viruses, several guidelines were obtained for the later investigation, in order to facilitate the attainment of the original objective, i.e. to determine the difference in chlorine resistance of the 25 viruses selected:

(a) The partially treated Potomac water contained a small amount of chemicals which adversely affected the chlorination process on viruses as compared with the demand-free water. Since the differences in virucidal effect between the two waters observed was small and a slightly slowed down reaction was desirable to observe the difference, if any, in chlorine resistance among viruses, the use of the Potomac water as the suspending medium in study of the remaining viruses was justifiable.

(b) One level of chlorine at 0.5 mg/l appeared adequate to test the remaining viruses, because a greater difference in devitalization rate among the viruses could be observed at this dose level than at higher levels of chlorine.

(c) By the same reason experiments carried out at 2°C gave more detailed information than those carried out at 22°C. Further, under the field conditions, the lowest water temperature encountered in winter seasons, in Washington, D.C. area, is about 2°C. Thus, it appeared more appropriate to conduct all later experiments at 2°C than at higher temperatures.

(d) Since a rapid rate of devitalization of the 6 viruses was obtained under these experimental conditions, more frequent sampling in the early part of the experiment became necessary in order to elicit more valid curves of inactivation for comparison.

(C) Determination of chlorine resistance of viruses in Potomac water: From the foregoing investigation, it was completely agreed between the Corps of Engineers and the NEWSL that the remaining viral strains should be tested under the following conditions:

(1) Use the Potomac H_2O as suspending medium.

(2) Use 0.5 mg/l of free chlorine.

(3) Test to be carried out at 2°C.

(4) Collect samples more frequently than in previous experiments.

To date, the remaining 19 viruses have been completed for testing under these conditions. The tested viruses were: poliovirus types 2 and 3; coxsackievirus types A5, A6, B2, B3, B4 and B5; echovirus 5,7,8,9.11,12 and 29; adenovirus types 7a and 12, and reovirus types 1 and 2. The experimental data are summarized in Tables 32 through 68.

As clearly shown in these tables, the residual free chlorine was successfully kept at the wanted level. Any adjustments were in general made in the early part of each experiment. As for the rates of viral devitalization, the kinetics were of the first order in the majority of cases. Several viruses showed a tailing off phenomenon which are further analyzed and discussed in a later section. By visual observation, the difference in resistance to chlorine exhibited by the 25 viruses is considerable. Further comparison is presented in the next section.

(D) The relative resistance of 25 viruses to chlorine in Potomac water:

From the data obtained with the 25 viruses tested, the degree of relative resistance to the disinfecting effect of 0.5 mg/l

free chlorine in the Potomac water is compared by using 3 parameters:

(1) The percentage of surviving virus at the end of 5 minutes of contact with chlorine;

(2) Minutes required to devitalize 99.99% of virus as demonstrated by the experiments;

(3) Minutes required to devitalize 99.99% of virus in the first order of kinetics by extrapolation from the above data.

The values calculated on the basis of the above criteria for all viruses tested are summarized in Table 69. The viruses are arranged according to the degree of resistance to chlorine in an ascending order, i.e. the least resistant virus appears at the top. In the first column are presented the 5 minute surviving values; in the second column, the extrapolated values and in the third column, the actual values obtained from experiments. Since the viruses were killed rapidly by chlorination, in most instances, only the 5 minute samples gave a measurable number of viruses. The weakness of this comparison, however, is that only one value from each experiment is used. This would be subject to larger errors if the one value was off from any experimental inaccuracy or assay variations. It is felt at this time that the comparison using the extrapolated numbers

of minutes to devitalize 4 log cycles of viruses is most reliable. The values are derived by extending the first order devitalization curve until reaching 4 log cycles below the initial virus titer. This is based on the assumption that the virus used for study is a genetically homogenous population and all are single particles. In this case, more experimental values from each curve are being used and only the first order kinetics are compared. In most instances, the minutes required to achieve 99.99% devitalization can be directly obtained from the experimental data. Only on five occasions are the observed values different from the extrapolated values. The discrepancy between the extrapolated values and the experimental results may be due to genetic variations of virus population in one stock; viral aggregates present in the preparation; etc. Further analysis and discussion of this problem are presented in the next section. It is also important to note that the great majority of viruses are placed in a proper rank for their chlorine resistance by using any of the three parameters.

When the five minute surviving values are compared, the type 1 echovirus appears to be most resistant. While using the other two parameters for comparison, this virus falls short in

its rank of resistance. This may be attributed to the fact that the virions per se exhibited a genetic characteristic of multiple hit inactivation or that the preparation used contained mostly viral aggregates. Further study of this interesting phenomenon is definitely indicated.

It is clear from the data in Table 69 that there is indeed a wide range of difference in chlorine resistance among the viruses tested. The time required to kill 99.99% of virus may vary from <2.8 minutes for a reovirus to 43.7 minutes for a coxsackievirus. This observation definitely has a direct bearing on practical application of the chlorination process to disinfect water for a number of purposes.

(E) The retardation phenomenon:

The possible causes for the phenomenon of retarded devitalization are:

(1) Presence of viral aggregates in the preparation tested;

(2) Inhomogeneity of viral population in which a fraction is genetically more resistant to chlorine than the great majority of viruses;

(3) Others.

It is understandable that a clump containing several virus particles would manifest as one virus under the current assay

conditions. These clumps, however, should be more resistant to chlorination than the single particles as long as one virus in this group remains alive. The procedures used in this study to exclude viral clumps included filtration through Millipore membrane or centrifugation at 10,000 rpm for one hour. After such treatments some preparations still contained a small number of aggregates probably from certain technical difficulties or any unobserved mechanical failures. This seemed to be the case for the five viruses whose inactivation curves showed an apparent retardation.

To determine the size of average clumps present in the particular preparations used for devitalization, Chang (35) proposed to employ the following formula:

$$(1)\quad K_1 = \frac{\text{Log } A_1 - \text{Log } B_1}{t_1}$$

$$(2)\quad K_2 = \frac{\text{Log } A_2 - \text{Log } B_2}{t_2}$$

$$(3)\quad \text{Size of clump} = \frac{K_1}{K_2}$$

A_1 = Virus titer at 0 time

B_1 = Virus titer where graphically shown the first order of kinetic ends

t_1 = Time in minutes from A_1 to B_1

A_2 = Virus titer at the beginning of t_2 period, generally is B_1

B_2 = Virus titer where the second slope ends

t_2 = The time in minutes from A_2 to B_2

By using the above formula, the size of viral clumps present in each preparation is estimated. The results for the coxsackievirus type B3, the echovirus type 12 and the adenovirus type 7a are illustrated in Figure 3, 4, and 5, respectively. As shown, the number of the clumped population in these preparations was quite small, i.e. 0.1% or less. On the average, one clump of the echovirus contained approximately 10 virions; and one clump of the adenovirus and the coxsackievirus, 20 virions.

As for the second possible cause, the genetically resistant viral population, in theory, can be isolated and identified by serial passages of the surviving virus after chlorination in appropriate tissue culture. Until this is done, however, there is no evidence to exclude this possibility. Other possible causes include, for example, virus embedded in cellular debris, coated by organic matter, etc. It is apparent at this time that further investigation is needed in order to clarify this problem. It is of practical importance to have these answers in order that a realistic guideline for chlorination practice

can be established.

(F) Isolation of virus from Potomac water:

An attempt was made to isolate human enteric virus from the raw and partially treated Potomac estuarine water and the finished water from the Washington, D.C. area. Two samples each, of the raw and treated Potomac water and one sample of the finished water, were collected during the period of January to February 1970. The volume of each sample was 20 liters. All samples were concentrated by a two-step procedure: (a) hydroextraction with polyethylene glycol to reduce the volume to approximately 4 liters. (b) two-phase polymer separation to further concentrate each sample to approximately 20 ml.

The concentrates were inoculated into (1) the primary humen embryonic kidney tissue culture bottles and (b) the primary African green monkey kidney culture bottles. These bottles were fed periodically and observed for 2-3 weeks. Regardless of the cellular changes observed, these bottles were frozen and thawed three (3) times and the contents were pooled according to groups. These pools were further passaged twice consecutively in the same tissue cultures. If no cytopathic effect (CPE) was observed for all 3 passages, the sample is considered as containing no detectable viral pollutant. If a typical CPE was observed the preparations was further

passaged in the tissue culture until an adequate titer was obtained. The isolates were identified by using type specific antisera. The results are summarized in Table 70.

As shown, one isolate was obtained from one raw water sample and another from the finished water sample which was collected from the faucet of the Washington Water Treatment Plant. Since the number of samples examined was very small, significance of this finding cannot be ascertained at this time. In addition, the possibility of these isolations as a result of laboratory contamination also cannot be excluded. It is apparent, however, that continued effort should be made in examining the water supplies in the Washington, D.C. area for viral pollutants.

DISCUSSION AND FUTURE CONSIDERATION

In the past two decades, 9 types of human enteric viruses were tested for their resistance to chlorine by a number of investigators. The viruses tested were poliovirus types 1,2, and 3; coxsackievirus types A2, A9, B1, and B5; echovirus type 7; and adenovirus type 3. By using a stool suspension, the resistance of infectious hepatitis virus to chlorine was also studied in human volunteers by Neefe et al (18). A direct comparison of the chlorine resistance cannot be made for these viruses, because these experiments were carried out using varying procedures and under widely different conditions. There is, however, indication that a considerable range of difference in resistance exists among the few viruses tested.

Generally speaking, the most viruses tested in the above studies were about 10x more resistant than the enteric bacteria with the exception that the type 3 adenovirus appeared even more sensitive to chlorine than were the bacteria. It was also shown that the efficacy of chlorination varied considerably under different physical and chemical conditions. It is of practical importance to point out that based solely on these limited data some public health authorities appear to be satisfied with the current water treatments and believe that these processes are capable of producing

drinking water free of viral pollutants. The weaknesses of these data in supporting the above contention may be listed below:

(a) Study of the chlorine resistance on only 10% of human enteric viruses is an insufficient sampling to establish a general pattern for all the viruses in these groups, especially since a wide range of resistance was observed.

(b) The tests were conducted under highly artificial conditions and on a laboratory scale. It is inappropriate to directly translate this information to field application.

(c) The viruses used for these experiments were laboratory strains except the hepatitis virus studied which was directly derived from a natural source, i.e. human feces. These former types of "laboratory virus" are probably different from the virus occurring in natural environments. For example, virus in nature may exist in varying sizes of clumps, may be embedded in cellular debris, coated with organic materials, etc. Thus, their resistance to chlorination may be considerably greater than the virus in laboratory preparations.

(d) The many environmental factors which may affect the efficiency of chlorination have not been thoroughly explored

and defined. The factors may include: turbidity, bacterial density, presence of a wide variety of inorganic and organic materials and even nonpathogenic microbes (amoeba, algae, etc.), meteorologic changes (sunshine, humidity, rainfall, etc.) and so on. The most effective conditions for chlorination have not been completely defined although low turbidity, low pH and several others were shown to be helpful for disinfection. The most unfavorable conditions are also not well known. The latter conditions may aid the natural forms of virus to survive through the various treatments and cause waterborne diseases.

For the above reasons, we are not at all sure today whether or not our current water treatment processes are capable of eliminating all harmful viral pollutants from all water supplies. Also we wonder whether or not these treatments will provide the public with a sufficient margin of safety to cope with occasional occurrence of very unfavorable conditions. These conditions, for instance, may include severe epidemics of human enteric virus disease, unfavorable hydrographical conditions, drought, cold weather and many others. As pointed out in the Background section, there is ample circumstantial evidence to suggest that the high annual incidence of infectious hepatitis may be partially attributable to a widespread and chronic pollu-

tion of water supplies with low levels of this virus.

While numerous investigations should be initiated in the laboratory as well as in the field to resolve these problems, the present study was aimed at shedding more light on one crucial problem in this area: is the current practice of chlorination foolproof in providing virus free water supplies? In order to obtain answers to this question, clarification is essential to the following two questions which are of a more basic nature:

(a) Is there a real species and/or strain difference in chlorine resistance among the human enteric viruses?

(b) What is the magnitude, if there is such a difference?

By testing a significant number (25%) of the known human enteric viruses under rigidly controlled conditions in the NEWS Laboratory, the following facts emerged:

(a) There is indeed a virus species (serologic types) difference in chlorine resistance.

(b) The difference is considerable under identical experimental conditions, e.g. 2.7 minutes were required to kill 99.99% of a reovirus whereas 43.7 minutes were required to kill the same amount of coxsackievirus.

It is apparent, however, that no definite pattern can be derived from these data at this time to categorize the relative

resistance of all existing types of enteric viruses. We cannot yet predict which virus will exhibit the greatest resistance. There is little doubt that the confidence on the effectiveness of chlorination process against virus can be raised by examining more strains of virus. The only way to secure a full assurance in this regard is to examine all known types and strains of human enteric viruses. To achieve this would require considerably more funding and time. It is felt, however, that such approach may be mandatory in the future because of the potentially serious health hazards associated with these viruses.

In addition, the current study should be expanded to include investigation of the following two aspects in order to make the data from this study more meaningful and applicable to the real world situation:

(a) Repeat those experiments in which an obvious retardation in viral devitalization was observed, and determine its cause.

(b) Explore the difference, if any, in resistance between the viruses prepared in the laboratory and those found in nature. For a preliminary study, the poliovirus of fecal origin should be tested for its chlorine resistance and directly compared with those propagated in monkey kidney tissue cultures.

It is felt that the results from the above investigations will at least lay a firm theoretical foundation for the application of chlorine to disinfect water supplies of various types and open new avenues for future investigation of a more comprehensive coverage on this subject. The investment involved in this phase of study is minimal when one considers the significance of the information which can be obtained on the transmission and eradication of human enteric virus diseases in this country.

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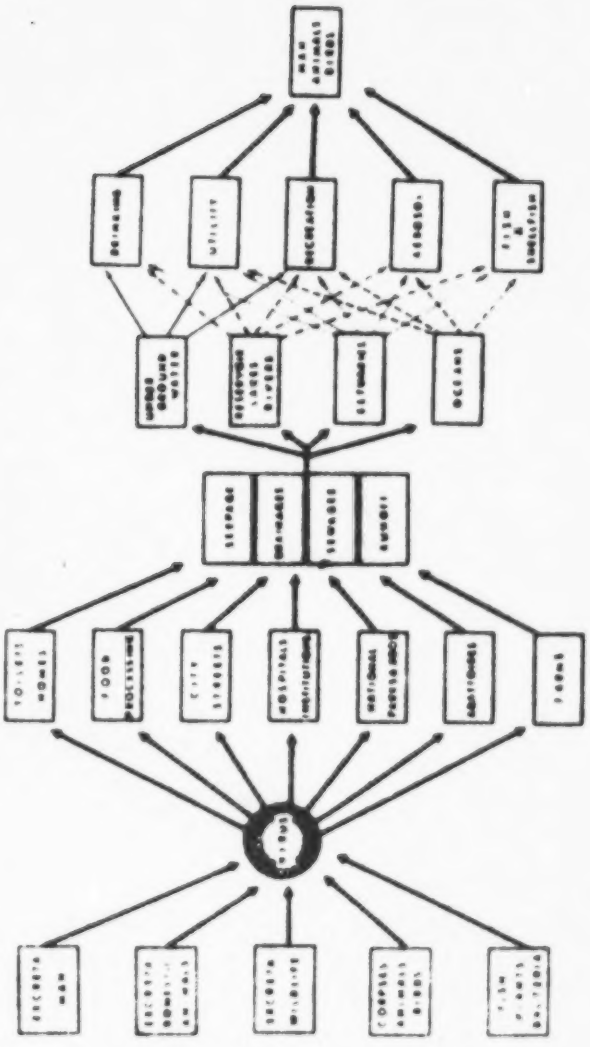
Figure 1



THE SOURCE & TRANSMISSION OF VIRUS THROUGH WATER ROUTES

Figure 2. Various modes of transmission of human enteric viruses

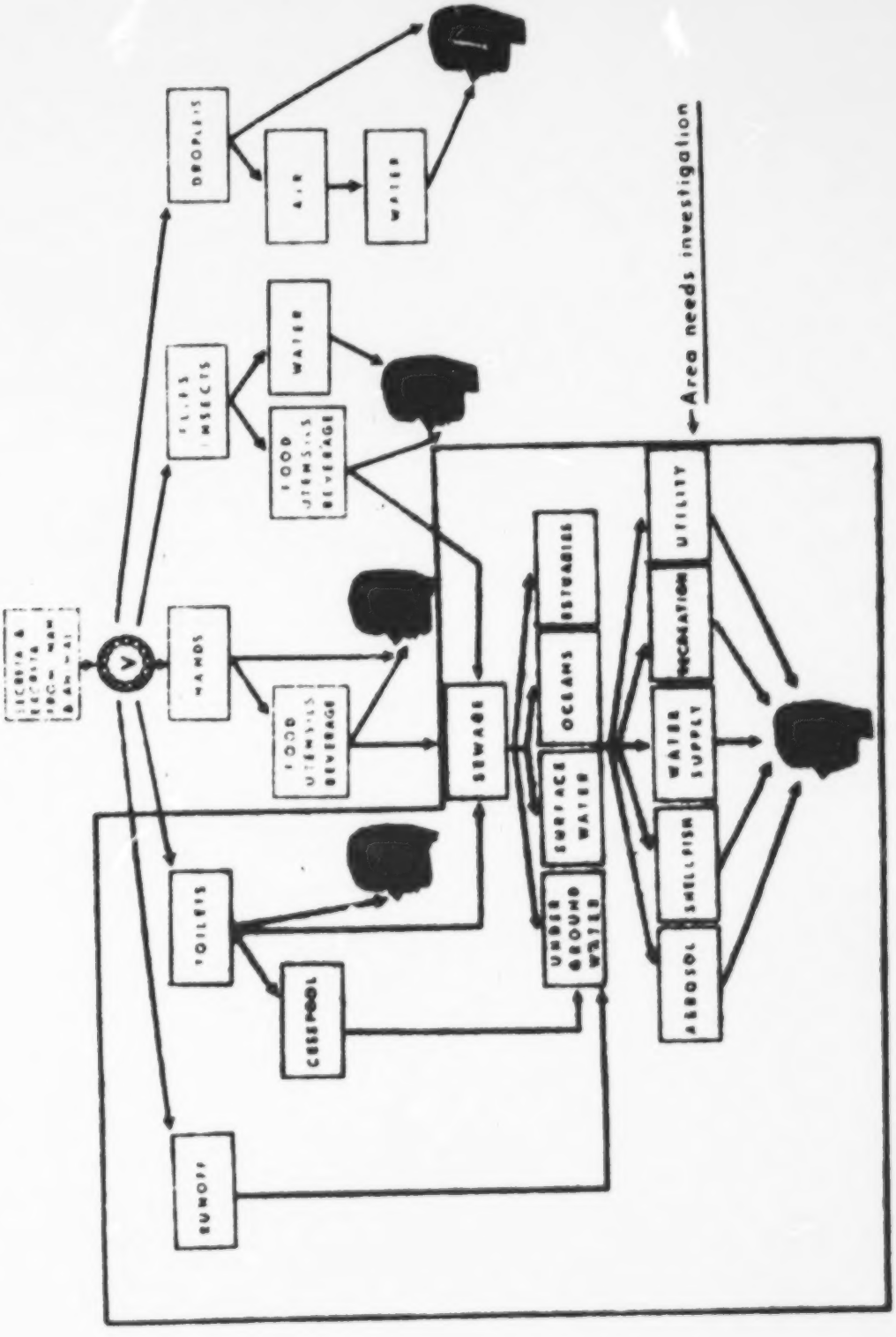


Figure 3

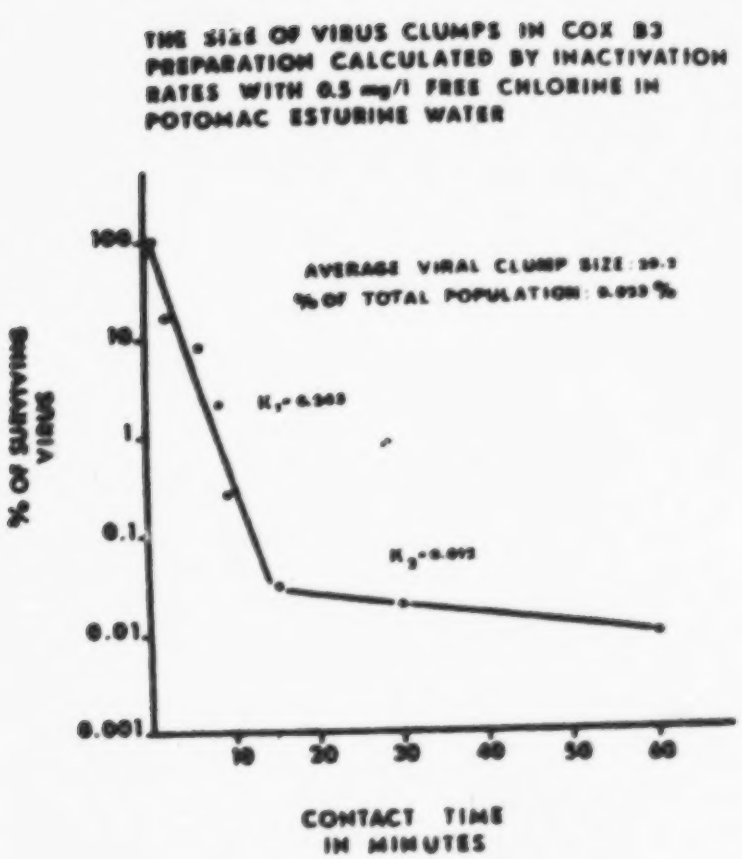

THE SIZE OF VIRUS CLUMPS IN COX B3 PREPARATION CALCULATED BY INACTIVATION RATES WITH 0.5 mg/l FREE CHLORINE IN POTOMAC ESTUARINE WATER
AVERAGE VIRAL CLUMP SIZE:
% OF TOTAL POPULATION:
100
10
1
0.1
0.01
0.001
% OF SURVIVING VIRUS
10
20
30
40
50
60
CONTACT TIME IN MINUTES

Figure 4

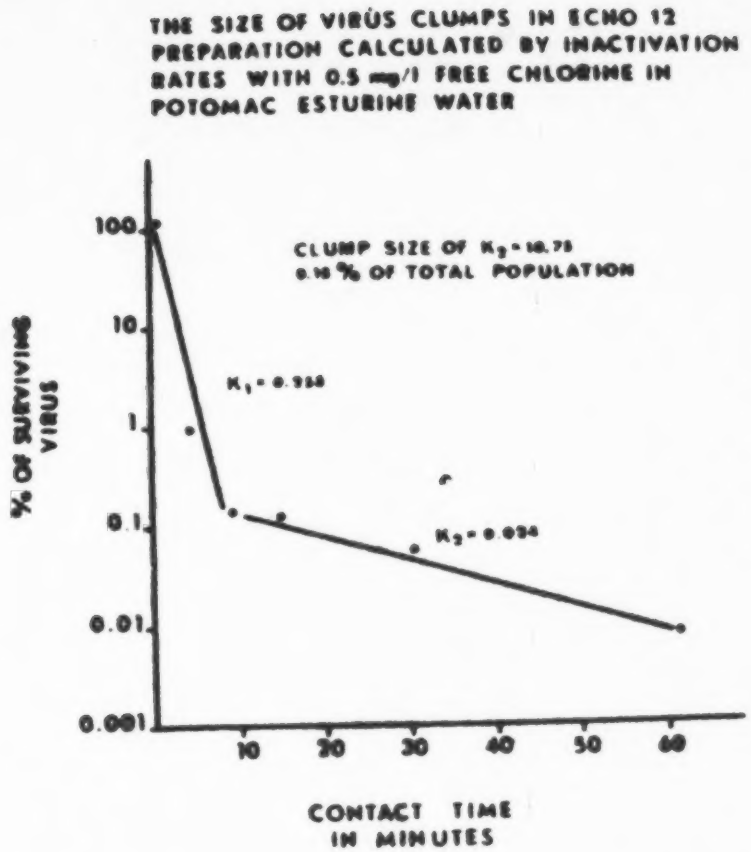

THE SIZE OF VIRUS CLUMPS IN ECHO 12 PREPARATION CALCULATED BY INACTIVATION RATES WITH 0.5 mg/l FREE CHLORINE IN POTOMAC ESTUARINE WATER
CLUMP SIZE OF K_2 = 10.75
0.10 % OF TOTAL POPULATION
K_1 = 0.938
K_2 = 0.034
% OF SURVIVING VIRUS
100
10
1
0.1
0.01
0.001
10
20
30
40
50
60
CONTACT TIME IN MINUTES

Figure 5

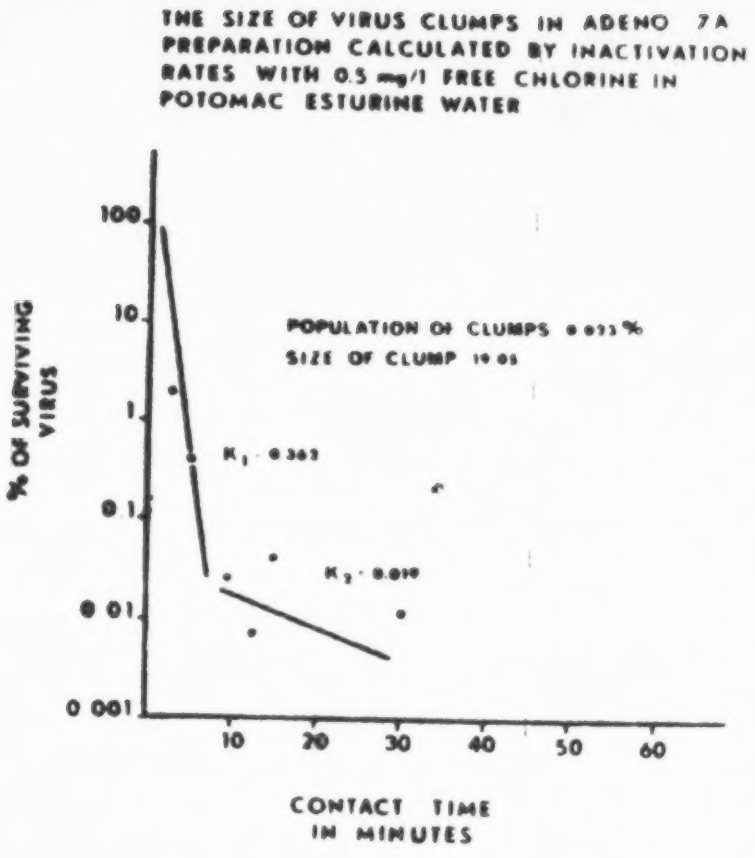

THE SIZE OF VIRUS CLUMPS IN ADENO 7A PREPARATION CALCULATED BY INACTIVATION RATES WITH 0.5 mg/l FREE CHLORINE IN POTOMAC ESTURINE WATER
% OF SURVIVING VIRUS
100
10
1
0.1
0.01
0.001
POPULATION OF CLUMPS 0.033 %
SIZE OF CLUMP 19.05
K1 = 0.363
K2 = 0.019
10
20
30
40
50
60
CONTACT TIME IN MINUTES

Table 1

The human enteric viruses which can be waterborne
(Wilner, 1966)

Group	Subgroup	# of types or subtypes	Type of nucleic acid	Size (diameter)	Reaction to	
					Ether	pH 3.0
Enterovirus	Poliovirus	3	RNA	28 mμ	Resistant	Stable
	Echovirus	31	"	"	"	"
	Coxsackievirus					
	A	24	"	"	"	"
	B	6	"	"	"	"
Reovirus		6	"	70 - 77 mμ	"	"
Adenovirus		31	DNA	60 - 85 mμ	"	"
Hepatitis		>1	?		"	"
Unknown		?				
Total		>102				

Table 2

Minimal infective doses of attenuated poliovirus
for human hosts by oral route
(Plotkin and Katz, 1967)

Hosts	Virus	Dose (PFU's)	Carrier rate	% infected
Adults	Poliovirus type 1 (SM strain)	200	4/4*	100
		20	4/4	100
		2	2/3	67
		0.2	0/2	0
Premature infants	Poliovirus type 3 (Fox strain)	10	2/3	67
		2.5	3/9	33
		1	3/10	30

* Number of persons developed into carrier for the virus/Number of persons who had taken the virus orally.

Table 3

Clinical syndromes associated with infections of human enteroviruses
(Kibrick, 1964)

Virus group	Types	Clinical syndrome
1) Poliovirus	1,2,3	Muscular paralysis Aseptic meningitis Febrile episodes
2) Coxsackie-virus A	1,2,3,4,5,6,8,10,22	Herpangina
	10	Acute lymphatic pharyngitis
	2,4,7,9,10	Aseptic meningitis
	7,9	Paralysis
	4,5,6,9,10	Exanthem (hand-foot-mouth disease)
	9,16	Pneumonitis of infants
	21,24	Common cold
	4,9	Hepatitis
	18,20,21,22,14	Infantile diarrhea
3) Coxsackie-virus B	1-5	Pleurodynia
	1-6	Aseptic meningitis
	2-5	Paralysis
	1-5	Meningoencephalitis & myocarditis
	1-5	Pericarditis & myocarditis
	4-5	URI & pneumonia
	5	Rash or hepatitis
4) Echovirus	1-34 except 12,24,26,29,32, 33,34	Aseptic meningitis
	2,4,6,9,11,30 or others	Paralysis
	2,6,9,19 or others	Guillain-Barre's syndrome
	2,4,6,9,11,16 18, etc.	Exanthem
	4,9,11,20,25,etc.	Respiratory disease
	Many types	Diarrhea
	1,6,9	Epidemic myalgia
	1,6,9,19	Pericarditis & myocarditis
	4,9	Hepatitis

Table 4

Recent reports on clinical illnesses associated with human enteric viruses (Liu, 1970)

Disease		Virus	Patients	Locale	References
(1)	Otitis conjunctivitis fatal pneumonia	Adeno 1,3, 6,7	16 infants	Moscow, U.S.S.R.	Oganasyan, O.T. Vop. Virus 12:582.
(2)	Acute appendicitis	Cox B and adeno	400+ cases all ages	Kyoto, Japan	Tobe, T. Lancet 1:1343, 1965.
(3)	Intussusception	Adeno	5-7 children	Formosa	Clark, Phillips & Alexander J.A.M.A. 208: 1671, 1969.
(4)	Subacute thyroiditis	Cox, Echo, adeno (Mumps, Infl.)	32 adults	Toronto, Canada	Volpe, R., et al J. Cl. End. 27: 1275, 1967.
(5)	Pericarditis myocarditis	Cox A9 Cox A9	3 young adults 9 young adults	Munich, Germany	Zollner, N., et al, Deutsch Med.
(6)	Interstitial myocarditis	Cox B	17 hearts from autopsy (to 30 years)	New Orleans, Louisiana	Burch, G.E., et al Am. J. Heart Dis. 75:13, 1967.
(7)	Valvulitis	Cox B	3 cases	?	?
(8)	Spontaneous abortion	Cox B	348 over normal average/month	Bornholm, Hungary	Czeizel, A. J.A.M.A. 201:142 1967.
(9)	Pericarditis	Cox B	12 adults 2 infants	Melbourne, Australia	Bennet, N.M., et al Am. H.J., 1967.
(10)	Meningitis	Mostly echo A9	132 cases	Vienna and lower Austria	Rahl, H. Munchen Med. Wschr. 109:1900, 1967.
(11)	Insulin-dependent diabetes	Cox B4	100 young adults	England	Gamble & Taylor B.M.J. 3:631, 1969.

Table 4. CONTINUED

Disease		Virus	Patients	Locale	References
(12)	Congenital heart anomalies	Cox B3 & 4	26 infants	U.S.A.	Brown & Evans, J.A.M.A. 199: 183, 1967.
(13)	Acute myocarditis (fatal)	Echo 9	34 yr. old dentist	New York, New York	Monit, G.R.G., et al, New Eng. J. Med. 277: 1353, 1967.
(14)	Acute myocarditis (fatal)	Echo 22	14 yr. old boy with agamma globulinemia	Los Angeles, California	Maller, H.M., et al J. Pediat. 71: 204, 1967.
(15)	Paralytic poliomyelitis	Vaccine poliovirus	29 yr. old male	Seattle, Washington	Swanson, et al J.A.M.A. 201:771, 1967.
(16)	Paralytic poliomyelitis	Vaccine strains	87 cases	U.S.A.	Henderson, et al J.A.M.A. 190:41-48, 1964.
(17)	Paralytic poliomyelitis (both lower ext.)	Vaccine poliovirus type 2	43 yr. old female	Worcester, Massachusetts	Morse, L.J., et al, J.A.M.A. 197: 1034, 1966.

Table 5

Reported hepatitis outbreaks from contaminated potable water
(1895 - 1970)

Type of water	Number of outbreaks	Hepatitis cases		Attack rate % (a)
		Total	Range	
1. Municipal system	19	31.352	21-28,745	0.1-32.3
2. Wells	10	762	9-348	9.3-60.8
3. Institutional system	7	717	20-260	0.7-32.4
4. Utility supply	4	102	14-50	1.6-50.0
5. Community supply	4	107	34-47	N.O. (b) (40.0) (c)
6. Private supply	3	174	41-77	11.4
7. Open spring	1	173	-	27.9
8. Irrigation ditch	1	72	-	N.O.
9. Stream	1	68	-	N.O.
10. Pond	1	21	-	N.O.
11. Contaminated bucket	1	16	-	21.1
12. River	1	6	-	31.6
Total	53	33,570	6-28,745	15.0(d)

(a) Unadjusted rate of persons presumably consuming the same water.

(b) N.O. - Not obtainable.

(c) One outbreak.

(d) Average attack rate excluding the New Delhi outbreak.

Table 6. Poliomyelitis outbreaks associated with various types of drinking water

	Year	Country	Place	Water supply	Number of cases	Number exposed	Attack rate %
1.	1944	Sweden	Town	Municipal system filtered surface water	65	13,000	0.5
2.	1944	Sweden	Town	Municipal system untreated deep well water	53	26,500	0.2
3.	1948	Sweden	Town	Municipal system untreated deep well water	63	31,500	0.2
4.	1949	Sweden	Malmoe	Municipal system filtered surface water	138	138,000	0.1
5.	1952	U.S.A.	Huskerville	Municipal system	45	652	6.7*
6.	1953	Canada	Edmonton	Municipal system chlorinated surface water	75	>75,000	<9.1
	Average				78.8	56,800	0.22
	Total				394	284,000	0.14

* This outbreak is excluded from computation for average attack rate because the water was known to be directly polluted with raw fecal materials from defective toilet systems.

Table 7

Germicidal effect of free chlorine in water reported in literature

	Microorganism	Temp. °C.	Final pH	Free Cl mg/liter	Destruction %/min.
1.	S. typhi in DFW	20-25	7.0	0.06	>99.99/5
		20-25	8.5	0.06	>99.99/5
2.	E. coli in DFW	20-25	7.0	0.04	>99.99/5
		20-25	8.5	0.07	>99.99/5
3.	A. aerogenes in DFW	20-25	7.0	0.05	>99.99/5
4.	Sh. dysenteriae in DFW	20-25	7.0	0.05	>99.99/5
5.	Infectious hepatitis	25	6.4-6.9	0.40	0.0/30
	Treated H_2O)	25		1.08	No disease/30
	Untreated H_2O)	25		15.23	Attenuated/30
6.	Purified polio 1	0	7.0	0.53	99.6/4.5
	(Mahoney) in DFW	0	8.5	5.0	99.6/3
		25-28	7.0	0.21-0.30	99.9/3
		25-28	9.0	0.21-0.30	99.99/8
	(MK500) in DFW	1-5	7.0	0.30	99/16
7.	Purified polio 2	1-5	7.0	0.23	99.9/16
	(MEF_1) in DFW	25-28	7.0	0.11-0.20	99.9/2
8.	Purified polio 3 (Saukett) in DFW	1-5	7.0	0.11-0.20	99.9/30
9.	Purified coxsackie	3-6	6.8-7.1	1.9-2.2	99.6/4
	A2 in DFW	3-6	8.8-9.0	7.4-8.3	99.6/5
		27-29	6.9-7.1	0.1-0.18	99.6/4
		27-29	8.8-9.0	0.92-1.0	99.6/3
10.	Purified coxsackie B1 (49683) in DFW	25-28	7.0	0.21-0.30	99.9/8
11.	Purified coxsackie	1-5	7.0	0.30	99.9/16
	B5 (EA80) in DFW	25-28	7.0	0.21-0.30	99.9/1
		25-28	9.0	0.21-0.30	99.9/8
12.	Purified adeno	25	6.9-7.1	0.20	99.8/8-16"
	type 3 in DFW	25	8.8-9.0	0.20	99.8/40-50"

Table 8

25 virus strains included for the chlorination study

	Group	Subgroup	Type	Strain	Assay	System
1.	Enterovirus	Poliovirus	1	LSc 2ab	Plaque	AGMK*
2.			2	CH 2ab	"	"
3.			3	Leon 2 ab	"	"
4.		Coxsackievirus	A5	Swartz	LD_{50}	Suckling Mice
5.			A6	Gdula	"	"
6.			A9	PB Bozek	Plaque	AGMK
7.			B1	Conn-5	"	"
8.			B2	Ohio-1	"	"
9.			B3	Nancy	"	"
10.			B4	JVB	"	"
11.			B5	Faulkner	"	"
12.		Echovirus	1	Farouk	"	"
13.			5	Noyce	"	"

* AGMK = Primary African green monkey kidney tissue culture.

** HEK = Primary human embryonic kidney tissue culture.

*** L929 = Secondary L929 mouse fibroblast tissue culture.

Table 8. CONTINUED

	Group	Subgroup	Type	Strain	Assay	System
14.			7	Wallace	Plaque	AGMK
15.			8	Bryson	"	"
16.			9	Vispo	"	"
17.			11	Gregory	"	"
18.			12	Travis	"	"
19.			29	JV-10	"	"
20.	Adenovirus		3	G.B.	$TCID_{50}$	HEK**
21.			7a	S-1058	"	"
22.			12	Huie	"	"
23.	Reovirus		1	Lang	Plaque	L-929***
24.			2	Jones	"	"
25.			3	Abney	"	"

Table 9. Protocol for testing 6 representative viruses under varying experimental conditions

Contact time (min)	Demand-free water										Partially treated Potomac					
	2°C					22°C					2°C					
	0 mg/l	0.5 mg/l		1,5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l
0	Virus assay	Resid. Cl.	Virus assay	Resid. Cl.	Virus assay	Virus assay	Resid. Cl.	Virus assay	Resid. Cl.	Virus assay	Virus assay	Resid. Cl.	-	Resid. Cl.	-	Assay
(15")	-	-	-	-	-	-	-	-	-	-	-	-	Virus assay	-	Virus assay	-
5	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
15	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
30	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
60	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
120	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"

rotocol for testing 6 representative viruses under varying experimental conditions

Demand-free water						Partially treated Potomac water									
	22°C					2°C					22°C				
1	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
Virus assay	Virus assay	Resid. Cl.	Virus assay	Resid. Cl.	Virus assay	Virus assay	Resid. Cl.	- Virus assay	Resid. Cl.	- Virus assay	Assay	Resid. Cl.	- Virus assay	Resid. Cl.	- Virus assay
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"

Table 10

Chemical characteristics of the treated Potomac estuarine water

		(1970) February Test	(1970) August Test	(1971) May Test
1.	Turbidity	0.35 J.T.U.	0.55 J.T.U.	0.85 J.T.U.
2.	pH	6.80	6.85	6.95
3.	Total dissolved solids	132.2 mg/l	139.5 mg/l	133.0 mg/l
4.	Alkalinity: (Phenolphthalein (Methyl orange	0	0 47.5 mg/l ($CaCO_3$)	0 48.2 mg/l ($CaCO_3$)
5.	Sodium	8.78	6.45	6.52
6.	Chloride	17.0	16.0	14.5
7.	Hardness ($CaCO_3$)	83.24 mg/l	79.5 mg/l	80.0 mg/l
8.	Phosphate (ortho)	0.368 mg/l (as PO_4)	0.368 mg/l (as PO_4)	0.332 mg/l (as PO_4)
9.	Nitrate	3.28 mg/l	3.88 mg/l	3.80 mg/l
10.	Sulfate	45.0 mg/l	48.0 mg/l	53.0 mg/l

Table 11

Effect of Cl on poliovirus type 1 at 2°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	5.10±0.79**					7.63±1.00				
(15")							4,330±750		257.00±50	
5		6.70±10.71	0.13	2.00±2.8	0.039		85.30±24.76	1.12	41.30±256	0.541
10		5.00±3.06	0.10	<0.17±0	<0.003		13.30±8.0	0.175	<0.17±0	<0.002
15	5.23±0.35	<0.17±0	<0.003			6.80±1.15	<0.17±0	<0.002		
30										
60	5.53±0.28					6.90±1.50				
120										

* % = % of surviving virus.

** ± one standard deviation.

Table 12

Effect of Cl on poliovirus type 1 in demand-free and Potomac water at 22°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	37.33±7.52**					34.67±6.88				
(15")							13.33±16.30		2.34+2.1	
5		<0.17±0	<0.005	<0.17±0	<0.005		2.00+2.70	0.06	1.34±1.70	0.04
15		<0.17±0	<0.005	<0.17±0	<0.005		<0.17±0	<0.005		
30	23.00±4.52					39.67±8.14				
120	28.33±7.10					34.00±7.00				

* % = % of surviving virus.

** ± one standard deviation.

Table 13

Free residual chlorine* (mg/l) throughout the inactivation experiments for poliovirus 1 (at pH 7.8)

Contact time (min)	Demand-free water				Potomac water			
	2°C		22°C		2°C		22°C	
	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg
0	0.52	1.53	0.55	1.60	0.36+	1.45+	0.42+	1.52
5	0.46+	1.45+	0.35+	1.50+	0.57	1.51	0.51	1.45
6	0.60	1.45	0.55	1.46+	0.52	1.45	0.44+	1.45+
30	0.60	1.55	0.55	1.53	0.52	1.50	0.51	1.42
60	0.60	1.52	0.56	1.45	0.56	1.52	0.51	1.42+
120	0.54	1.55	0.62	1.50	0.66	1.51	0.50	1.45

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 14

Effect of Cl on coxsackievirus A9 at 2°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	91.70±5.80**					103.00±11.08				
(15")							1970.00±750			
5		17.00±10.09	0.019	<0.17±0	<0.0002		163.50+6	0.16	0.17±0	0.00016
10		<0.17±0	<0.0002	<0.17±0	<0.0002		<0.17±0	<0.00016	<0.17±0	<0.00016
15	89.70±7.10	<0.17±0	<0.0002			101.30±23.30	<0.17±0	<0.00016		
30										
60	82.70±9.2					83.00±11.64				
120										

* % = % of surviving virus.

** ± one standard deviation.

Table 15

Effect of Cl on coxsackievirus type A9 at 22°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml (x 10^3)	PFU/ml	%*	PFU/ml	%	PFU/ml (x 10^3)	PFU/ml	%	PFU/ml	%
0	89.30±12.18**					80.70±40.80				
(15")							8730±1764		4.75±4.86	0.0059
5		6.83±3.46	0.008	<0.17±0	<0.0002		1966±532	2.437	<0.17±0	<0.0002
15		<0.17±0	<0.0002	<0.17±0	<0.0002		<0.17±0	0.0002		
30	50.00±8.86	<0.17±0	<0.0002			69.00±24.00	<0.17±0	<0.0002		
120	15.00±3.94					48.00±11.52				

* % = % of surviving virus.

** ± one standard deviation.

Table 16

Free residual chlorine* (mg/l) throughout the inactivation experiments for coxsackievirus A9 (at pH 7.8)

Contact time (min)	Demand-free water				Potomac water			
	2°C		22°C		2°C		22°C	
	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg
0	0.50	1.50	0.55	1.52	0.32+	1.30+	0.50	1.50
5	0.37+	1.48	0.50	1.47	0.15+	1.40+	0.25+	1.42+
15	0.58	1.49	0.45+	1.45+	0.52	1.48	0.53	1.55
30	0.55	1.48	0.40+	1.55	0.53	1.45	0.52	1.46
60	0.55	1.47	0.50	1.52	0.52	1.46	0.51	1.44
120	0.55	1.48	0.55	1.55	0.53	1.46	0.51	1.46

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 17

Effect of Cl on coxsackievirus B1 at 2°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	10.13±0.9**					8.47±0.39				
(15")										
5		348.35±27.82	3.44	4.66±3.10	0.046		1060.00±93.17	12.52	40±10	0.48
10		<0.17±0	<0.0017	<0.17±0	<0.0017		<0.17±0	<0.002	<0.17±0	<0.002
15	7.67±1.96	<0.17±0	<0.0017			12.80±3.18	<0.17±0	<0.002	<0.17±0	<0.002
30										
60	7.47±1.55					11.00±2.59				
120										

* % = % of surviving virus.

** ± one standard deviation.

Table 18

Effect of Cl on coxsackievirus B1 at 22°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	31.50±5.09**					21.17±4.45				
(15")							5667.00±362.00	26.77	1193.40±253.00	5.63
5		790.00±89.78	251	<0.17±0	<0.0005		630.00±180.00	3.26	<0.17±0	<0.0008
15		<0.17±0	<0.0005				<0.17±0	<0.0008		
30	31.17±3.17					21.17±3.49				
60										
120	25.84±3.44					22.67±2.07				

* % = % of surviving virus.

** ± one standard deviation.

Table 19

Free residual chlorine* (mg/l) throughout the inactivation experiments for coxsackievirus B1 (at pH 7.8)

Contact time (min)	Demand-free water				Potomac water			
	2°C		22°C		2°C		22°C	
	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg
0	0.51	1.52	0.55	1.55	0.12+	1.10	0.06+	1.50
5	0.06+	1.45+	0.35+	1.35+	0.38+	1.35	0.25+	1.42+
15	0.63	1.50	0.53	1.48	0.52	1.50	0.59	1.42+
30	0.60	1.50	0.52	1.41+	0.35+	1.50	0.50+	1.52+
60	0.55+	1.48	0.32+	1.20+	0.53	1.50	0.30+	1.35+
120	0.12	1.46	0.66	1.48	0.50	1.49	0.52	1.52

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 20

Effect of Cl on echovirus type 7 at 2°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	9.20±3.14**					7.87±2.38				
(15")							4668.00±1632.00	59.31	<0.17±0	<0.002
5		<0.17±0	<0.002	<0.17±0	<0.002		10.66±6.30	0.14	<0.17±0	<0.002
15		<0.17±0	<0.002				<0.17±0	<0.002		
30	9.47±309					7.73±1.49				
60										
120	7.47±2.00					4.67±3.20				

* % = % of surviving virus.

** ± one standard deviation.

Table 21

Effect of Cl on echovirus type 7 at 22°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml (x 10^3)	PFU/ml	%*	PFU/ml	%	PFU/ml (x 10^3)	PFU/ml	%	PFU/ml	%
0	3.80±0.42**					2.48±0.24				
(15")									<0.17±0	<0.0069
5		<0.17±0	<0.0045	<0.17±0	<0.0045		<0.17±0	<0.0069	<0.17±0	<0.0069
15		<0.17±0	<0.0045	<0.17±0	<0.0045		<0.17±0	<0.0069	<0.17±0	<0.0069
30	3.24±0.51					2.33±0.19				
60										
120	3.47±0.57					2.00±0.50				

* % = % of surviving virus.

** ± one standard deviation.

Table 22

Free residual chlorine* (mg/l) throughout the inactivation experiments for echovirus 7 (at pH 7.8)

Contact time (min)	Demand-free water				Potomac water			
	2°C		22°C		2°C		22°C	
	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg	0.5 mg	1.5 mg
0	0.5	1.50	0.5	1.5	0.025+	1.55	0.5	1.55
5	0.3	1.30+	0.3	1.35	0.21+	1.50	0.48	1.50
15	0.36+	1.45+	0.25	1.25	0.35+	1.48	0.45	1.42
30	0.46+	1.50	0.22	1.22	0.45+	1.46	0.40+	1.40+
60	0.50	1.50	0.22	1.22	0.45+	1.50	0.42+	1.55
120	0.50	1.50	0.12	1.11	0.45	1.37	0.45	1.38

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 23

Effect of Cl on adenovirus type 3 in demand-free and Potomac water at 2°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	TCD_{50}/ml*	TCD_{50}/ml	%**	TCD_{50}/ml	%	TCD_{50}/ml	TCD_{50}/ml	%	TCD_{50}/ml	%
0	4.50±1.17***					3.92±1.22				
5	4.18±1.59	<0.82±0	<0.002	<0.82±0	<0.002	3.91±1.33	<0.82±0	<0.008	2.13±1.27	1.62
15	4.10±1.44	<0.82±0	<0.002	<0.82±0	<0.002	3.96±1.33	<0.82±0	<0.008	<0.82±0	<0.008
30	3.85±1.42	<0.82±0	<0.002			4.92±1.26	<0.82±0	<0.008	<0.82±0	<0.008
60	4.12±1.43					4.06±1.39				
120	4.29±1.09					3.89±1.39				

*TCD_{50}/ml in log 10.

** % = % of surviving virus.

*** ± one standard deviation.

Table 24

Effect of Cl on adenovirus type 3 in demand-free and Potomac water at 22°C (pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	TCD_{50}/ml*	TCD_{50}/ml	%**	TCD_{50}/ml	%	TCD_{50}/ml	TCD_{50}/ml	%	TCD_{50}/ml	%
0	4.29±1.26***					5.05±1.47				
5	4.63±1.33	<0.82	<0.0034	<0.82	<0.0034	4.07±1.46	<0.82	<0.0006	<0.82	<0.0006
15	4.78±1.25	<0.82	<0.0034	<0.82	<0.0034	4.71±1.47	<0.82	<0.0006	<0.82	<0.0006
30	4.82±1.32	<0.82	<0.0034	<0.82	<0.0034	4.72±1.19	<0.82	<0.0006	<0.82	<0.0006
60	5.33±1.29					5.26±1.11				
120	4.44±1.51					4.55±1.29				

*TCD_{50}/ml in log 10.

** % = % of surviving virus.

*** ± one standard deviation.

Table 25

Free residual chlorine* (mg/l) throughout the inactivation experiments with adenovirus type 3 (at pH 7.8)

Contact time (min)	Free residual chlorine (mg/l)			
	Demand-free water		Potomac water	
	2°C	22°C	2°C	22°C
0	0.50	0.85	0.46	0.32+
5	0.15+	0.65	0.22+	0.61
15	0.35+	0.52	0.52	0.49+
30	0.50	0.52	0.52	0.58
60	0.50	0.52	0.55	0.47+
120	0.50	0.27	0.52	0.55

* The values were determined by using an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates points at which additional chlorine was added to bring the free residual back to 0.5 mg/l.

Table 26

Effect of Cl on reovirus type 3 at 2°C
(pH 7.8)

Contact time (min)	Demand-free H_2O					Potomac H_2O				
	0 mg/l	0.5 mg/l		1.5 mg/l		0 mg/l	0.5 mg/l		1.5 mg/l	
	PFU/ml ($\times 10^3$)	PFU/ml	%*	PFU/ml	%	PFU/ml ($\times 10^3$)	PFU/ml	%	PFU/ml	%
0	40.00±2.14**					35.00±5.28				
5		0.33±0.41	0.0008	<0.33±0	<0.0008		<0.33±0	<0.001	<0.33±0	<0.001
15	45.00±4.93	2.00±0.89	0.005	<0.33±0	<0.0008	35.00±5.28	<0.33±0	<0.001	<0.33±0	<0.001
30		<0.33±0	<0.0008							

* % = % of surviving virus.

** ± one standard deviation.

Table 27

Effect of Cl on reovirus type 3 in demand-free and Potomac water at 22°C
(pH 7.8)

Contact time (min)	Demand-free H_2O			Potomac H_2O		
	0 mg/l	0.5 mg/l		0 mg/l	0.5 mg/l	
	PFU/ml ($\times 10^2$)	PFU/ml	%*	PFU/ml ($\times 10^2$)	PFU/ml	%
0	49.00±4.45**			47.00±2.94		
5		<0.33±0	<0.0006		<0.33±0	<0.0006
15	52.00±4.7	<0.33±0	<0.0006	44.00±4.50	<0.33±0	<0.0006

* % = % of surviving virus.

** ± one standard deviation.

Table 28

Free residual chlorine* (mg/l) throughout the inactivation experiments with reovirus type 3 (at pH 7.8)

Contact time (min)	Free residual chlorine (mg/l)			
	Demand-free water		Potomac water	
	2°C	22°C	2°C	22°C
0	0.52	0.50	0.32+	0.40+
5	0.25+	0.18+	0.50+	0.53
15	0.46+	0.59+	0.45+	0.45+
30	0.51	0.63	0.52	0.50
60	0.52	0.63	0.52	0.52
120	0.51	0.40	0.52	0.52

* The values were determined by using an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates points at which additional chlorine was added to bring the free residual back to 0.5 mg/l.

Table 29

Effect of temperature on chlorination effect

Virus	Water	% of surviving virus after 5 min contact					
		0.5 mg/l			1.5 mg/l		
		2°C	22°C	Dif***	2°C	22°C	Dif
Polio 1	DFW*	0.130	<0.005	<0.125	0.039	<0.005	>0.034
	Potomac**	1.118	0.060	1.058	0.541	0.040	0.501
Cox A9	DFW	0.019	0.008	0.011	0.0002	<0.0002	-
	Potomac	0.161	2.437	-2.276	<0.0002	<0.0002	-
B1	DFW	3.439	2.508	0.931	0.046	<0.0005	>0.0455
	Potomac	12.515	3.259	9.256	0.480	<0.008	>0.472
Echo 7	DFW	<0.002	<0.005	-	<0.002	<0.005	-
	Potomac	<0.135	<0.007	0.128	<0.007	<0.002	-
Adeno 3	DFW	<0.002	<0.003	-	<0.002	<0.003	-
	Potomac	<0.008	<0.001	-	1.620	<0.0006	>1.6194
Reo 3	DFW	0.008	<0.006	>0.002	-	-	-
	Potomac	<0.001	<0.001	-	<0.001	-	-

* DFW = demand free water.

** Potomac = partially treated Potomac River estuarine water.

*** Dif = Difference between value at 2°C and value at 22°C.

Table 30

Influence of water used on chlorination study

Virus	Chlorine mg/l	% of surviving virus after 5 min contact					
		2°C			22°C		
		DFW*	Potomac**	Dif***	DFW	Potomac	Dif
Polio 1	0.5	0.130	1.118	0.988	<0.005	0.060	>0.055
	1.5	0.039	0.541	0.502	<0.005	0.040	>0.035
Cox A9	0.5	0.019	0.161	0.142	0.008	2.437	2.429
	1.5	0.0002	<0.002	-	<0.0002	<0.0002	-
B1	0.5	3.439	12.515	9.076	2.508	3.259	0.851
	1.5	0.046	0.480	0.434	<0.0005	<0.008	-
Echo 7	0.5	<0.002	0.135	>0.132	<0.005	<0.007	-
	1.5	<0.002	<0.007	-	<0.005	<0.002	-
Adeno 3	0.5	<0.002	<0.008	-	<0.003	<0.001	-
	1.5	<0.002	1.620	>1.618	<0.003	<0.0006	-
Reo 3	0.5	0.008	<0.001	->0.007	<0.006	<0.001	-
	1.5	<0.001	<0.001	-	-	-	-

*DFW = demand free water.

**Potomac = partially treated Potomac estuarine water.

***Dif = Difference between the value of Potomac and the value of DFW.

Table 31

Effect of chlorine dose on viral inactivation

Virus	Water	% of surviving virus after 5 min contact					
		2°C			22°C		
		0.5 mg	1.5 mg	Dif***	0.5 mg	1.5 mg	Dif
Polio 1	DFW*	0.130	0.039	0.091	<0.005	<0.005	-
	Potomac**	1.118	0.541	0.567	0.060	0.040	0.020
Cox A9	DFW	0.019	0.0002	0.0188	0.008	<0.0002	0.0078
	Potomac	0.161	<0.0002	0.1608	2.437	<0.0002	2.4368
B1	DFW	3.439	0.046	3.393	2.508	<0.0005	>2.5075
	Potomac	12.515	0.480	12.035	3.259	<0.008	>3.251
Echo 7	DFW	<0.002	<0.002	-	<0.005	<0.005	-
	Potomac	0.135	<0.007	>0.128	<0.007	<0.002	-
Adeno 3	DFW	<0.002	<0.002	-	<0.003	<0.003	-
	Potomac	<0.008	1.620	->1.612	<0.001	<0.0006	-
Reo 3	DFW	0.008	<0.001	>0.007	<0.006	-	-
	Potomac	<0.001	<0.001	-	<0.001	-	-

* DFW = demand free water.

** Potomac = partially treated Potomac estuarine water.

*** Dif = the difference between the value obtained at 0.5 mg/l and the value obtained at 1.5 mg/l.

Table 32

Effect of Cl on poliovirus type 2 in Potomac water
(2^{o}C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l	
		PFU/ml	%*
0	55.00±21.67**		
(15")	62.50±7.80	5570±834.00	89.12
5		1880±305.50	30.08
10		502±71.90	1.92
15	48.30±19.40	262±42.60	1.91
20		48±4.90	1.49
30	52.00±17.30	7.30±1.60	0.99
40		2.30±0.30	0.04
60	53.00		

* % = % of surviving virus.

** ± one standard deviation.

Table 33

Free residual chlorine* (mg/l) throughout the inactivation experiment for poliovirus 2 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.45+
5	0.40+
10	0.30+
15	0.45+
30	0.50
60	0.45

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 34

Effect of Cl on poliovirus type 3 in Potomac water
(2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($\times 10^2$)	0.5 mg/l	
		PFU/ml	%*
0	14.33±2.42**		
(15")		603±111	
2.5		74.66±9.26	5.35
5	14.16±3.56	34.66±7.20	2.42
7.5		18.33±3.88	1.28
10		9.00±2.36	0.63
15		17.50±2.34	1.22
30	15.50±4.03	<0.17±0	<0.01
60		<0.17±0	<0.01
120	10.67±2.80		

* % = % of surviving virus.

** ± one standard deviation.

Table 35

Free residual chlorine* (mg/l) throughout the inactivation experiment for poliovirus 3 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.55
5	0.55
15	0.50
30	0.55
60	0.50
120	0.55

*Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

Table 36. Effect of Cl on coxsackievirus type A5 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l $LD_{50/ml}$ (Log_{10})	0.5 mg/l $LD_{50/ml}$ (Log_{10})	0.5 mg/l % *
0	4.95±0.37**		
5		4.22±0.36	18.624
10		3.82±0.35	7.585
15	4.55±0.61	3.19±0.20	1.738
30		1.59±0.57	0.044
60		0.75±0.22	0.006
120		0.52±0.60	0.003

* % = % of surviving virus.

** ± one standard deviation.

Table 37

Free residual chlorine* (mg/l) throughout the inactivation experiment for coxsackievirus type A5 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.40+
5	0.55
10	0.50
15	0.50+
30	0.55
60	0.55
120	0.50

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 38

Effect of Cl on coxsackievirus type A6 in Potomac water
(at 2°C and pH 7.8)

Contact time	0 mg/l	0.5 mg/l	
	LD_{50}/ml (Log_{10})	LD_{50}/ml (Log_{10})	%*
0	5.63±0.49**		
(15")		4.99±0.26	22.906
5		4.69±0.04	11.481
10	5.41±0.14	4.85±0.12	16.593
15		4.30±0.03	4.676
30		4.16±0.03	3.387
60		4.11±0.19	3.019

* % = % of surviving virus.

** ± one standard deviation.

Table 39

Free residual chlorine* (mg/l) throughout the inactivation experiment for coxsackievirus type A6 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.60
5	0.58
10	0.53
15	0.52
30	0.50
60	0.45+
120	0.40

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 40. Effect of Cl on coxsackievirus type B2 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l PFU/ml	0.5 mg/l %*
0	23.00±12.65**		
(15")		108.00±86.6	4.696
5		1.75±1.90	0.076
10		<0.17±0	<0.008
15	27.30±2.81	<0.17±0	<0.008
30		<0.17±0	<0.008
60	23.80±4.02		

* % = % of surviving virus.

** ± one standard deviation.

Table 41

Free residual chlorine* (mg/l) throughout the inactivation experiment for coxsackievirus type B2 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.5
5	0.5
10	0.5
15	0.48
30	0.48
60	0.45
120	0.45

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

Table 42

Effect of Cl on coxsackievirus type B3 in Potomac water
(2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($\times 10^2$)	0.5 mg/l PFU/ml	0.5 mg/l %*
0	22.16±2.04**		
(15")		790.00±96	
2.5		276.70±47	12.482
5.0	23.00±3.35	161.70±18	7.297
7.5		45.00±26	2.031
10		5.17±3	0.233
15		0.50±2	0.025
30	20.16±2.64	0.33±2	0.015
60	21.67±4.94	<0.17±0	<0.008
120		<0.09±0	<0.004

* % = % of surviving virus.

** ± one standard deviation.

Table 43

Free residual chlorine* (mg/l) throughout the inactivation experiment for coxsackievirus B3 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.38+
5	0.45+
10	0.45+
15	0.55
30	0.50
60	0.50
120	0.50

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 44. Effect of Cl on coxsackievirus type B4 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l	
		PFU/ml	%*
0	25.67±2.25**		
(15")		206.70±38.85	8.052
5		3.34±2.73	0.126
10		<0.17±0	<0.007
15	28.17±5.19	<0.17±0	<0.007
30		<0.17±0	<0.007
60			
120	27.17±2.23		

* % = % of surviving virus.

** ± one standard deviation.

Table 45

Free residual chlorine* (mg/l) throughout the inactivation experiment for coxsackievirus type B4 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.6
5	0.58
10	0.56
15	0.55
30	0.50
60	0.45+
120	0.55

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 46

Effect of Cl on coxsackievirus type B5 in Potomac water
(2^{o}C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml (x 10^2)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	97.50±13.70**		
(15")		5446±588	53.682
2.5		5234±1210	53.659
5.0	77.50±9.85	1417±448	14.533
10		1342±215	13.764
15		270±88	2.769
30		10±5	0.103
60		<0.17±0	<0.002
120	11.65±605	<0.17±0	<0.002

* % = % of surviving virus.

** ± one standard deviation.

Table 47

Free residual chlorine* (mg/l) throughout the inactivation experiment for coxsackievirus B5 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.5+
5	0.52+
10	0.58
15	0.55
30	0.51
60	0.45+
120	0.47

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 48. Effect of Cl on echovirus type 1 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	48.00±13.26**		
(15")		2600.00±1872.00	54.167
2.5			
5.0		2740.00±313.00	57.083
10		1867.00±396.00	38.917
15	57.33±6.52	308.00±64.00	6.417
30		<0.08	<0.002
60			
120	46.00±8.29		

* % = % of surviving virus.

** ± one standard deviation.

Table 49

Free residual chlorine* (mg/l) throughout the inactivation experiment for echovirus type 1 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.30+
5	0.40+
10	0.45+
15	0.45+
30	0.40+
60	0.60
120	0.65

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 50. Effect of Cl on echovirus type 5 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($\times 10^2$)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	85.32±8.60**		
(15")		300.00±244.00	3.516
2.5		63.00±25.80	0.738
5.0		5.00±5.47	0.059
7.5		1.33±0.52	0.016
10.0		0.33±0.52	0.004
15.0	81.66±24.82	<0.17±0	<0.002
30.0		0.67±0.86	0.008

* % = % of surviving virus.

** ± one standard deviation.

Table 51

Free residual chlorine* (mg/1) throughout the inactivation experiment of echovirus type 5 in Potomac water at pH 7.8 and 2°C

Contact time (min)	0.5 mg
0	0.30+
5	0.50+
10	0.20+
15	0.10+
20	0.60
30	0.55
60	0.50
120	0.40

* Free residual chlorine determined by an amperometric titrator (Fischer and Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 52. Effect of Cl on echovirus type 8 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	85.34±2.73**		
(15")		6167.00±786.00	72.264
2.5			
5.0		473.33±59.50	5.546
7.5			
10		23.67±4.67	0.277
15	79.66±7.40	0.17±0.41	0.002
20		<0.17	<0.002
60	71.00±13.84		
120			

* % = % of surviving virus.

** ± one standard deviation.

Table 53

Free residual chlorine* (mg/1) throughout the inactivation experiment of echovirus type 8 in Potomac water at pH 7.8 and 2°C

Contact time (min)	0.5 mg
0	0.40+
5	0.35+
10	0.45+
15	0.52+
30	0.52
60	0.45+
120	0.48

* Free residual chlorine determined by an amperometric titrator (Fischer and Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 54. Effect of Cl on echovirus type 9 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l PFU/ml	0.5 mg/l %*
0	148.30±44.00**		
(15")		2850.00±575.00	19.218
2.5			
5.0		280.00±38.98	1.887
7.5			
10		30±2.52	0.202
15	163.30±41.30	0.17±0.41	0.001
30		<0.17	<0.001
60			
120	138.30±48.00		

* % = % of surviving virus.

** ± one standard deviation.

Table 55

Free residual chlorine* (mg/1) throughout the inactivation experiment of echovirus type 9 in Potomac water at pH 7.8 and 2°C

Contact time (min)	0.5 mg
0	0.35+
5	0.40+
10	0.52
15	0.50
30	0.45+
60	0.50
120	0.50

* Free residual chlorine determined by an amperometric titrator (Fischer and Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 56. Effect of Cl on echovirus type 11 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($x10^2$)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	67.20±7.69**		
(15")		7967.00±2443.00	118.557
5		1400.00±58.32	20.833
10		5.66±3.45	0.084
15	62.17±4.92	0.33±0.52	0.005
30		<0.17±0	<0.003
60	59.40±13.00		
120	62.00±21.24		

* % = % of surviving virus.

** ± one standard deviation.

Table 57

Free residual chlorine* (mg/l) throughout the inactivation experiment of echovirus type 11 in Potomac water at pH 7.8 and 2°C

Contact time (min)	0.5 mg
0	0.25+
5	0.50+
10	0.50
15	0.50
30	0.50
60	0.50
120	0.47

* Free residual chlorine determined by an amperometric titrator (Fischer and Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 58. Effect of Cl on echovirus type 12 in Potomac water (at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($\times 10^3$)	0.5 mg/l PFU/ml	0.5 mg/l % *	1.5 mg/l PFU/ml	1.5 mg/l %
0	4.73±0.71**				
(15")		713.20±99.20		125.00±35.35	
5		817.00±44.80	17.273	119.00±1.40	0.402
10		8.33±9.83	0.176		
15		6.33±3.72	0.134	2.50±1.40	0.053
30	5.90±2.03	3.33±1.63	0.070	0.50±1.40	0.011
60	4.60±1.11	0.50±1.08	0.011		
120		<0.17±0	<0.003		

* % = % of surviving virus.

** ± one standard deviation.

Table 59

Free residual chlorine* (mg/l) throughout the inactivation experiment for echovirus 12 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	0.5 mg
0	0.45+
5	0.51+
10	0.58
15	0.55
30	0.50
60	0.45
120	0.43

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to the desired level.

Table 60

Effect of Cl on Echovirus Type 29 in Potomac Water
(at 2°C. and pH 7.8)

Contact Time (Min)	0 mg/l PFU&ml ($\times 10^2$)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	78.35±42.00**		
(15'')		1066.00±796	
2.5		8.17.00±204	10.43
5		130.00±58	1.66
10		13.00±1	0.17
15	37.50±12.15	5.20±2	0.07
30		<0.17±0	<0.002
60	45.00±13.50	<0.17±0	<0.002

*% of Surviving Virus

**One Standard Deviation

Table 61

Free Residual Chlorine* (mg/1) Throughout the Inactivation Experiment for Echovirus 29 in Potomac Water (at pH 7.8 and 2°C.)

Contact Time (Min)	0.5 mg
0	0.40+
5	0.41+
10	0.40+
15	0.55
30	0.45+
60	0.50
120	0.50

* Free residual chlorine determined by an amperometric titrator (Fisher & Porter, Warminster, Pennsylvania).

\+ Indicates point at which additional chlorine was added to bring the free residual back to desired level.

Table 62

Effect of Cl on Adenovirus Type 7a in Potomac Water
(pH 7.8)

Contact Time (Min)	2°C. 0 mg/l TCD50/ml (Log 10)	2°C. 0.5 mg/l TCD50/ml (Log 10)	2°C. 0.5 mg/l % *	22°C. 0 mg/l TCD50/ml (Log 10)	22°C. 0.5 mg/l TCD50/ml (Log 10)	22°C. 0.5 mg/l %
0	4.29±1.11**			4.22±1.35		
2.5		2.39±1.19	1.26		1.79±1.50	0.37
5.0	4.39±1.27	1.81±1.05	0.33	3.88±1.27	1.17±1.32	0.09
7.5		1.82±1.33	0.33		0.76±0.18	0.03
10.0		0.67±1.91	0.02		<0.63***	<0.03
12.5		0.21***	0.01		<0.63	<0.03
15.0	4.63±1.33	1.01	0.05	4.13±1,27	<0.63	<0.03
20.0		<0.63	<0.02		<0.63	<0.03
25.0		<0.63	<0.02		<0.63	<0.03
30.0	4.62±1.18					
60.0	5.50±0.00					

* % of Surviving Virus.
** One Standard Deviation.
*** The Values are too low for Determination of Standard Deviation by Calculator.

Table 63

Free Residual Chlorine* (mg/l) Throughout the Inactivation Experiments for Adenovirus 7a in Potomac Water (at pH 7.8 and 2°C.)

Contact Time (Min)	Free Chlorine (mg/l) at 2°C.	Free Chlorine (mg/l) at 22°C.
0	0.50	0.50
5	0.45+	0.50+
10	0.45+	0.42+
15	0.48	0.57
30	0.49	0.58
60	0.50	0.58

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates point at which additional chlorine was added to bring the free residual back to 0.5 mg/l.

Table 64

Effect of Cl on Adenovirus Type 12 in Potomac Water
(pH 7.8)

Contact Time (Min)	2°C.			22°C.		
	0 mg/l	0.5 mg/l		0 mg/l	0.5 mg/l	
	TCD50/ml (Log 10)	TCD50/ml (Log 10)	% *	TCD50/ml (Log 10)	TCD50/ml (Log 10)	%
0	4.69±1.12**			4.50±1.13		
2.5		2.63±1.17	0.91		1.62±1.14	0.13
5.0	4.76±1.12	2.21±1.34	0.33	4.31±1.12	0.92±0.64	0.03
7.5		3.15±1.02	2.88		1.46±1.07	0.09
10.0		1.17±1.15	0.03		1.33±1.32	0.07
12.5		1.02±0.78	0.02		0.78±***	0.02
15.0	4.82±1.19	<0.63***	<0.01	4.65±1.17	<0.63	<0.01
20.0		<0.63	<0.01		<0.63	<0.01
25.0					<0.63	<0.01
30.0				5.17±1.33		
60.0	4.88±1.27			4.63±0.00		

* % of Surviving Virus.
** One Standard Deviation.
*** The Values are too low for Determination of Standard Deviation by Calculator.

Table 65

Free residual chlorine* (mg/l) throughout the inactivation experiments for adenovirus type 12

Contact time (min)	mg/l of free chlorine at 2°C	mg/l of free chlorine at 22°C
0	0.41	0.50
5	0.24+	0.45+
10	0.42+	0.50
15	0.57	0.50
30	0.58	0.55
60	0.58	0.52

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates points at which additional chlorine was added to bring the level back to 0.5 mg/l

Table 66

Effect of Cl on reovirus type 1 in Potomac water
(2^{o}C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($\times 10^2$)	0.5 mg/l PFU/ml	0.5 mg/l % *
0	62.66±6.88**		
(15")		0.66±0.52	
5	97.66±5.63	<0.33±0	<0.005
15	103.33±8.28	<0.33±0	<0.005

* % = % of surviving virus.

** ± one standard deviation.

Table 67

Effect of Cl on reovirus type 2 in Potomac water
(at 2°C and pH 7.8)

Contact time (min)	0 mg/l PFU/ml ($\times 10^3$)	0.5 mg/l PFU/ml	0.5 mg/l %*
0	34.33±2.56**		
2.5		40.00±4.29	0.117
5.0	35.66±2.69	0.66±0.52	0.002
7.5		0.33±0	<0.001
10.0		<3.34±0.82	0.009
12.5		<0.33±0	<0.001
15.0	29.00±4.18		

* % = % of surviving virus.

** ± one standard deviation.

Table 68

Free residual chlorine* (mg/l) throughout the inactivation experiments for reovirus types 1 and 2 in Potomac water (at pH 7.8 and 2°C)

Contact time (min)	Free residual chlorine (mg/l)	
	Reovirus 1	Reovirus 2
0	0.51	0.50
5	0.45	0.45+
10	0.42	0.40+
15	0.41+	0.46+
30	0.46+	0.52
60	0.55	0.52

* Free residual chlorine determined by an amperometric titrator (Fischer & Porter, Warminster, Pennsylvania).

+ Indicates points at which additional chlorine was added to bring the free residual back to 0.5 mg/l

Table 69. Relative resistance of 25 human enteric viruses to 0.5 mg/l free chlorine in Potomac water (pH 7.8 and 2°C)

	Comparison based on					
	5' survivors		First order reaction		Experimental	
	Virus	%	Virus	Min	Virus	Min
1.	Reo 3	<0.0009	Reo 1	2.7	Reo 1	2.7
2.	2	0.002	3	<4.0	3	<4.0
3.	1	<0.005	2	4.2	2	4.2
4.	Adeno 3	<0.008	Adeno 3	4.8	Adeno 3	<4.3
5.	Echo 5	0.059	Cox B2	6.5	Cox B2	6.5
6.	Echo 7	0.135	Cox A9	6.8	Cox A9	6.8
7.	Cox A9	0.161	Cox B4	7.0	Cox B4	7.0
8.	Adeno 7a	0.330	Echo 7	7.1	Echo 7	7.1
9.	Adeno 12	0.330	Echo 5	8.0	Echo 5	8.0
10.	Polio 1	1.118	Cox B1	8.5	Cox B1	8.5
11.	Echo 29	1.660	Echo 9	12.0	Echo 9	12.0
12.	Echo 9	1.887	Adeno 7a	12.5	Adeno 7a	12.5
13.	Polio 3	2.420	Echo 8	13.0	Echo 8	13.0
14.	Cox B2	4.696	Adeno 12	13.5	Echo 11	14.0
15.	Echo 8	5.546	Echo 11	14.0	Polio 1	16.2
16.	Cox B3	7.297	Echo 12	14.5	Echo 29	20.0
17.	Cox B4	8.052	Polio 1	16.2	Adeno 12	23.5
18.	Cox A6	11.481	Cox B3	16.2	Echo 1	27.0
19.	Cox B1	12.515	Polio 3	16.7	Polio 3	30.0
20.	Cox B5	14.533	Echo 29	20.0	Cox B3	35.0

Table 69. (Continued)

21.	Echo 12	17.273	Echo 1	27.0	Cox A5	35.3
22.	Cox A5	18.624	Cox A5	35.3	Cox B5	39.5
23.	Polio 2	20.000	Cox B5	39.5	Polio 2	40.0
24.	Echo 11	20.833	Polio 2	40.0	Echo 12	>60.0
25.	Echo 1	57.083	Cox A6	43.7	Cox A6	>120.0

Table 70

Examination of water supplies from Washington, D.C. area for viral pollutants

Date collected	Code	Source	Status	Presence of virus	Identification
2-9-70	W69	Potomac estuarine water	Raw	+	Reovirus
2-9-70	W70	"	Treated	0	
3-13-70	W71*	"	Raw	0	
3-13-70	W72	"	Treated	0	
3-13-70	W73	D.C. water treatment plants	Finished	+	Poliovirus & reovirus

* Heavily contaminated.